A SERIES OF HISTIDINELESS MUTANTS OF NEUROSPORA CRASSA

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LEIN, MITCHELL and HOULAHAN (1948) have reported the isolation of a histidine-requiring mutant of Neurospora by means of a variation in the technique described by BEADLE and TATUM (1945). They also found evidence that histidineless mutants had not previously been obtained because they were inhibited by substances present in the various media used for their isolation.

Since this time, six other histidineless mutants have been isolated in this laboratory using the technique described by LEIN *et al.* The present report is concerned with investigations on the biochemistry and genetics of these seven histidineless Neurospora mutants that have now been obtained. Isolation numbers of the mutants are C84, C85, C91, C94, C140, C141 and T1710. The seven mutants represent at least four different genetic loci as will be demonstrated. All are inhibited in the same manner as the strain reported previously.

EXPERIMENTAL

Histidine requirement

Stock cultures of the histidineless mutants are kept on minimal medium (RYAN, BEADLE and TATUM, 1943) supplemented with L-histidine since the mutants fail to grow on media containing hydrolyzed casein, yeast extract, liver extract, autolysate of Neurospora mycelium or corn meal infusion. (C140, C141, and T1710 were tested only on media containing hydrolyzed casein, yeast extract or autolysate of Neurospora mycelium. They will not grow on these media.)

The quantity of L-histidine hydrochloride monohydrate required for half-maximum growth by C84, C91, and C94 is 0.5 mg in 20 ml of medium. C85 and C140 require only about half this amount, while C141 requires slightly more (about 0.6 mg in 20 ml medium). T1710 needs only 0.09 mg for half-maximum growth. All of the mutants except C141 give straight-line growth curves which indicate a regular increase in growth with an increase in the amount of histidine supplied up to the point of maximum growth. C141, however, gives an S-shaped curve and shows no appreciable growth when less than 0.2 mg of histidine in 20 ml medium is furnished. Histidine requirements

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were determined by measuring the dry weights of mycelia obtained from cultures grown for 4 days at 25°C in 125-ml Erlenmeyer flasks containing 20 ml of basal medium supplemented with varying amounts of L-histidine HCl.

Other compounds have been tested for growth-stimulating properties on C84 and C94. Of those tested none has been found to promote growth of these mutants. Those compounds found inactive are imidazole, imidazole acrylic acid, imidazole lactic acid, histamine, histidinol, imidazole glyoxylic acid, methyl imidazole, imidazole carboxylic acid, imidazole aldehyde, 4-amino-5-imidazole carboxamide, 4-amino-5-imidazol carboxamidine, imidazole methanol, thiol-histidine, 1-methyl histidine, adenine, cytidine, DL-alanine, glycine, DL-serine, L-proline, L-oxyproline, L-aspartic acid, L-glutamic acid, guanidine and glycocyamine, as well as the amino acids DL-leucine, L-isoleucine, DL-valine, DL-phenylalanine, L-tryptophan, L-lysine, L-arginine, DL-methionine and DL-threonine. Roughly half of these amino acids are slightly stimulatory in the presence of histidine.

Inhibition

As investigations of biochemical mutants proceed it becomes increasingly obvious that acquisition by mutation, of a new growth requirement is often accompanied by other changes in the physiological behavior of the organism. One such change leads to inhibition of the mutant by normal metabolites in concentrations which do not affect the growth of the wild type (DOERMANN 1946) (TEAS, HOROWITZ and FLING 1949). This is the case with this series of histidineless mutants.

It was found that 5 mg of "Vitamin Free" casein acid hydrolysate (Nutritional Biochemical Corp.) brought about complete inhibition in the presence of 0.5 mg of L-histidine HCl. The amino acids known to be in casein, when tested singly in 2-mg quantities with the above amount of histidine, produced no inhibition, but a mixture of these was inhibitory. By testing various combinations it was found that if either L-arginine or L-lysine is present, then any one of the following will be inhibitory: L-leucine, DL-isoleucine, DL-valine, DL-methionine, glycine, L-tyrosine, DL-phenylalanine, L-tryptophan or histamine. No inhibition by the following compounds has been observed: DL-serine, DL-threonine, L-proline, L-oxyproline, L-cystine, L-aspartic acid, L-glutamic acid, tyramine, ethylamine, alanine, imidazole and imidazole acrylic acid. The above compounds, both active and inactive, were tested in the presence of tyrosine to determine whether they could replace lysine or arginine. None of them did so, nor did DL-citrulline, guanidine or glycocyamin. DL-Ornithine and α-amino-ϵ-guanidino caproic acid, however, were found to be effective.

Extensive tests with mutant C94 have provided quantitative data on the inhibitory ratios of histidine, arginine and tyrosine. In order for complete inhibition to take place the molar concentration of tyrosine must exceed that of histidine, but if tyrosine is increased then the arginine concentrations may

be much lower than that of histidine. Arginine alone produces about half inhibition in a molar concentration which is 7 times the histidine concentration, but increasing arginine to make a 14-fold difference has no further effect. Inhibition by tyrosine alone has not been observed.

Inhibitory combinations other than tyrosine and arginine have been tested less thoroughly, but tests which have been made show that with tyrosine, either lysine or ornithine has about the same activity as arginine. The tests indicate no great difference in effectiveness of the compounds which replace tyrosine. Concentrations necessary for complete inhibition vary with the age of the culture from which the inoculations are made, higher concentrations being required if very young cultures (two or three days old) are used. Also the inhibition is not nearly so great when the mutants are grown on solid agar medium which contains the inhibitors.

Experiments designed to test whether the inhibitions of histidine mutants, described above, are due to the action of modifier genes suggest that this is the case but the genetic analysis is not yet complete.

Genetic analysis

Six of the histidineless mutants were found among 1760 selected mutant spores (LEIN *et al.* 1948) isolated from the crosses of wild-type 7A with wild-type 8a after irradiating one or the other with ultraviolet. All wild-type crosses do not seem to produce viable histidineless mutants. For example, 1633 selected single ascospore isolates from two crosses between Abbott 4A and 8a yielded no histidineless mutants.

Tests for formation of heterocaryons between the mutants were carried out (BEADLE and COONRADT 1944). Flasks containing 20 ml basal medium supplemented with 0.01 mg of histidine HCl per ml of medium were inoculated with each mutant separately and in all possible combinations of two. C85 and C91 were not included. The amount of histidine added permits germination of the conidia of the above strains but is insufficient for much growth. It was found that heterocaryons are formed in all cases except in combinations between C94, C140 and T1710 and between C94 and C84.

This experiment provided evidence that at least three different genetic loci are involved. This was first verified by counting random spore types from crosses of each mutant with every other mutant. Ascospores, when ripe, were collected on solid minimal medium in Petri plates, heat activated at 60°C for 30 minutes and then incubated overnight at 25°C. The plates were then examined for wild-type and histidineless spores. Mutant spores germinate on this medium but the hyphae do not grow whereas growth of the wild-type continues until the plate is covered. Since both members of each cross are histidineless mutants, any wild-type spore must be the result of a genetic recombination which eliminated both histidineless genes of the parents. These genes must, therefore, have different loci. Counts of wild-type and mutant spores from each cross yielded evidence that mutants C84, C94, C140 and C141 are genetically different. C84 behaves as an allele of C85 and C91, and

C140 as an allele of T1710. The latter two mutants, C140 and T1710 are linked to C94.

The results obtained from the above experiments were confirmed by dissecting asci from each of the crosses between histidineless mutants. All 8 spores from an ascus were removed in order and placed in a row on a Petri plate of solid minimal medium, 30 to 50 rows per plate. The spores were activated, allowed to germinate and grow overnight and then they were examined. Classes of asci recorded were those having eight histidineless spores, those having six histidineless and two wild, and those having four histidineless and four wild. Pertinent data obtained from these crosses are given in table 1. The results show that the mutant genes of C84 and C141 are not linked to each other or to C94, C140 or T1710. The last three do show linkage and

TABLE 1

Genetic analysis of histidineless mutants by dissection of asci progeny from intercrosses.

Cross	Ascus types*						Total No. asci	Linkage
	8H		6H–2+		4H–4+			
	Number	Percent	Number	Percent	Number	Percent		
C84 × C94	10	24.4	24	58.5	7	17.1	41	Not linked
C84 × C140	13	30.5	27	45.8	14	23.7	59	Not linked
C84 × C141	20	24.1	47	56.7	16	19.2	83	Not linked
C84 × T1710	6	22.2	13	48.2	8	29.6	27	Not linked
C94 × C140	104	88.1	13	11.0	1	0.9	118	Linked
C94 × C141	22	29.0	48	63.2	6	7.8	76	Not linked
C94 × T1710	113	85.6	19	14.4	0	0.0	132	Linked
C140 × C141	23	22.0	62	59.6	19	18.4	104	Not linked
C140 × T1710	135	100.0	0	0.0	0	0.0	135	Linked
C141 × T1710	24	25.5	63	67.1	7	7.4	94	Not linked

* 8H = asci having 8 histidineless spores; 6H–2+ = asci having 6 histidineless spores and 2 wild-type spores; 4H–4+ = asci having 4 histidineless spores and 4 wild-type spores.

the genetic evidence suggests that the mutant genes of C140 and T1710 are alleles. It should be noted, however, that observations of aborted spore patterns (McCLINTOCK 1945) with crosses involving T1710 indicate the existence of a chromosomal aberration in this strain. As will be described later in this report, evidence indicates that mutants T1710 and C140 are physiologically different. Thus the four mutants C84, C94, C140 and C141 are genetically different and T1710 may represent a fifth type. Mutants C84, C141 and those of the linked group (C94, C140 and T1710) have been crossed to marker mutants and the histidineless mutants placed in the linkage groups described by HOULAHAN *et al.* (1949). Pertinent data are included in table 2. Strain C84 can thus be placed in group *E*, C141 in group *D*, and the remaining three strains in group *A*.

In the course of the genetic experiments described above double histidineless mutants involving C84, C94, C140, C141 and T1710 were isolated. All

were verified by outcrossing them to wild type and examining asci. Any ascus from such an outcross which has a 6 histidineless–2 wild or an 8 histidineless composition confirms the fact that the histidineless parent is a double mutant.

From each outcross of the double mutants the spores of several asci having the 6 histidineless–2 wild composition were transferred to tubes (containing basal agar medium supplemented with histidine) and retained for other tests.

Accumulations

By application of the Pauly reaction for detection of histidine, as modified by JORPES (1932), it has been demonstrated that mutants C84, C85, C91, C141 and T1710 accumulate in the culture medium substances that appear to be imidazole derivatives. C94 and C140 show no significant accumulations

TABLE 2

*Linkage of histidineless mutants to other Neurospora mutants.**

Histidine mutant	Linkage group	Marker	Wild type spores	Mutant spores	Percent wild type
C84	B	51602	381	837	31.2
C84	C	10575	454	1129	28.6
C84	E	33933	304	1387	14.2
C84	E	37401	133	3991	3.2
C94	A	35203	67	1645	3.9
C94	B or D	45502	359	959	27.2
C94	C	10575	153	400	27.6
C94	E	33933	353	1024	26.7
C141	B	75001	730	2222	24.7
C141	B	51602	1013	2787	26.6
C141	C	10575	375	1190	23.9
C141	D	37301	681	4210	13.9
C141	D	38502	320	2128	13.1
C141	E	33933	438	1396	23.9

* See HOULAHAN, BEADLE and CALHOUN (1949) for identification of linkage groups and marker mutants.

of substances that give this color reaction nor do wild type strains. All of the mutants were allowed to grow for 4 days at 25°C with a quantity of histidine that will give half maximum growth. These accumulations were confirmed by chromatographing on filter paper by the method of WILLIAMS and KIRBY (1948) culture filtrates of each of the mutants. Substances were located on the chromatographs by the AMES and MITCHELL (1951) modification of the Pauly test. The solvent used for chromatographing was 3 parts propanol to 1 part 0.2 normal ammonium hydroxide.

According to the chromatographic evidence C84, C85 and C91 all accumulate the same compound, and since genetic tests have indicated that these three mutants are due to mutations at the same locus, only C84 was tested further. The C84 compound becomes purple-red on developing and has an Rf of 0.46. C141 accumulates two compounds one of which is apparently the same as that of C84. The second compound becomes a bright red on developing and

has an Rf of 0.73. The T1710 compound becomes a bright red and has an Rf of 0.65. All of the above compounds were found to be inactive biologically when tested for growth-promoting activity on the mutants. However, they proved to be extremely valuable in determining the possible sequence of biosynthetic reactions interfered with by the mutants.

None of the mutants was observed to accumulate any substance in the medium which will stimulate the growth of any other histidineless mutant. This has been tested by incorporating the filtrate of a mutant, grown on half its maximum requirement of histidine, into basal medium and inoculating with each of the other mutants. It has also been tested by chromatographing concentrated filtrates of the mutants and eluting segments of the chromatograph with basal medium. The fractions were then tested as growth supplements for all of the mutants.

Biochemical evidence for the sequence of genes in histidine biosynthesis

Previous investigations in this laboratory have made use of double mutants to provide evidence of a biochemical sequence for Neurospora mutants that have the same growth requirement (MITCHELL and HOULAHAN 1946). In the present case it has been demonstrated that mutants C84, C141 and T1710 accumulate substances that appear to be imidazole derivatives while mutants C94 and C140 do not accumulate such substances. If the five mutants correspond to five steps in a sequence of biochemical reactions that leads to the formation of histidine, and if the substances accumulated are intermediates or closely related to intermediates, then double mutants should have the properties of the single mutant that corresponds to the earlier chemical reaction in the sequence. For example the double mutant C94/C141 should not accumulate an imidazole derivative if C94 corresponds to an earlier biochemical reaction than that of C141. In order to test the above hypothesis the following series of experiments was carried out.

The double mutants and each of the single mutants were cultured for 4 days in 125-ml flasks on 20 ml minimal medium supplemented with histidine. Since the half-maximum histidine requirement of the double mutant was unknown, two cultures of each double mutant were used. These were supplemented with the half-maximum histidine requirement of each of the single mutant parents. After four days growth at 25°C a 1-ml sample was removed from each flask and transferred to a test tube. A standard set of test tubes containing minimal medium supplemented with 25γ, 50γ, and 100γ of L-histidine HCl per ml was also prepared. The intensities of the colors from all samples, after developing with the diazo reagent, were immediately read in the Beckman spectrophotometer at a wave length of 480 mμ. The compounds accumulated by the mutants give characteristic intensities of color when developed by this test.

Another series of 1-ml samples corresponding to those used in the above test was withdrawn from the growth flasks, evaporated to 0.1 ml over sulfuric acid *in vacuo* and chromatographed in the manner previously mentioned. All

samples were placed on the same sheet of filter paper to make comparison easier.

The mycelial pad from each of the growth flasks was then removed, dried at 80°C for 5 hours and weighed to determine the growth of each mutant on each histidine level in 4 days.

TABLE 3

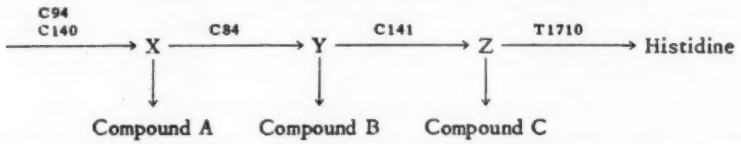
Comparison of growth and products accumulated by different histidineless Neurospora mutants.

Histidineless mutant	Amt. histidine (mg/ml) in medium before growth	Dry weight of mycelium in mg	Optical density	Compound accumulated (chromatograph)
Minimal (blank)	....	....	0.0	....
" + 25γ histidine	....	....	0.270	....
" + 50γ "	....	....	0.575	....
" + 100γ "	....	....	1.210	....
7A (wild type)	0.0045	80.9	0.000	
" " "	0.015	79.8	−0.020	None
" " "	0.030	85.5	0.000	
C84 (single)	0.0045	5.8	0.225	
" "	0.015	15.0	0.480	Type A
" "	0.030	27.5	0.480	
C94 (single)	0.0045	5.8	0.000	
" "	0.015	20.1	0.000	None
" "	0.030	35.5	0.016	
C140 (single)	0.0045	6.4	0.017	
" "	0.015	19.3	0.083	None
" "	0.030	34.1	0.090	
C141 (single)	0.0045	0.2	0.025	
" "	0.015	10.0	0.690	Types A and B
" "	0.030	18.0	1.620	
T1710 (single)	0.0045	60.5	0.250	
" "	0.015	61.8	0.182	Type C
" "	0.030	64,4	0.185	
C84/C94 (double)	0.015	18.6	0.075	None
C84/C140 "	0.015	18.0	0.072	None
C84/C141 "	0.015	0.2	0.118	Type A
" "	0.030	8.6	0.620	
C84/T1710 "	0.0045	6.6	0.160	Type A
" "	0.015	18.2	0.550	
C94/C140 "	0.015	12.9	0.071	None
C94/C141 "	0.015	18.9	0.050	None
" "	0.030	33.7	0.060	
C94/T1710 "	0.0045	5.2	0.065	None
" "	0.015	14.9	0.014	
C140/C141 "	0.015	15.6	0.034	None
" "	0.030	31.2	0.070	
C141/T1710 "	0.0045	10.4	0.275	Types A and B
" "	0.030	32.4	0.780	

The same series of experiments was repeated using a 10-day growth period. The results of the above experiments are presented in table 3. The results for the 4-day growth series are not given since they were much like those obtained at 10 days.

It will be noted in table 3 that wild-type Neurospora metabolizes all of the histidine in the medium even though this supplement is not needed for its growth. The controls also show that the histidineless mutants use all of the histidine in the medium and that their growth is dependent on the amount of histidine present.

On the basis of the results of table 3 it is postulated that the sequence of reactions interfered with by the mutants is as follows:



The results of the chromatographic and colorimetric determinations on any given double mutant are consistent in all cases with the placing of a single mutant character in the above scheme. C94 and C140 appear to correspond to biochemical reactions that come first since any double mutant made with either does not accumulate any imidazole-like substance. It is not possible to determine the order with respect to C94 and C140 since neither is an accumulator. C84 is placed before C141 and T1710 since the double mutants C84/C141 and C84/T1710 accumulate only the compound accumulated by C84. C141 is placed before T1710 since the double mutant C141/T1710 accumulates the compounds of C141 and C84. This is characteristic of the C141 single mutant. The dry weights of the mycelia show that all of the double mutants take the histidine requirement of one single, double mutant C94/T1710 that of C94, the double mutant C84/C141 that of C141, etc.

As a further check on this experiment each double mutant used previously was outcrossed to wild-type. After the spores had ripened a number of asci from each outcross were dissected on minimal agar Petri plates. The composition of each ascus as to histidineless and wild-type spores was determined as described earlier. Several asci having a 6 histidineless–2 wild spore composition were saved from every cross. One spore from each of the four spore pairs of such asci was transferred from the minimal agar plate to a test tube containing histidine-supplemented medium. Each ascus of this type provides one wild-type culture, one double mutant culture, and cultures of each of the parental single mutants. The same tests as those previously carried out with the double and single mutants were performed on each of the four spore pairs of the asci saved from the outcrosses. Several asci from each outcross except C84/C140, C84/T1710 and C94/T1710 were analyzed in this manner.

The results obtained from the above experiment demonstrate that the strains tested as double mutants (table 3) did have the expected genetic con-

stitution. The data on accumulation of imidazole-like substances by each culture from each ascus tested are completely consistent with the results shown earlier in table 3. Thus all of the data are in accord with the biochemical sequence that has been proposed. This is true even though the compounds accumulated by the single mutants do not seem to be intermediates in biosynthesis of histidine but appear to be by-products of these biosynthetic reactions.

SUMMARY

A series of 7 *Neurospora crassa* mutants which require histidine for growth have been isolated using the procedure of LEIN *et al.* (1948). These have been given isolation numbers C84, C85, C91, C94, C140, C141, and T1710.

No compound other than histidine has been found to be active in promoting growth, but different mutants require different amounts of histidine.

Growth of all of these mutants is inhibited, even when histidine is furnished, if mixtures of certain amino acids are present in the culture medium. When any one of L-leucine, DL-isoleucine, DL-valine, DL-methionine, glycine, L-tyrosine, DL-phenylalanine, L-tryptophan or histamine is present along with L-arginine, DL-ornithine, α-amino ε-guanidino caproic acid or L-lysine inhibition results. Tests on inhibitory concentrations of tyrosine and arginine indicate that the molar concentration of tyrosine must exceed that of histidine for complete inhibition, but if tyrosine is increased then the arginine concentrations may be lower than that of histidine.

A genetic analysis of the 7 mutants indicates that 3 different linkage groups and 4 different loci are involved. C84, C85 and C91 are in one linkage group and appear to be alleles. C94, C140 and T1710 are in another linkage group, but C94 is at a different locus from that of C140 and T1710 which act as alleles. However, the latter two mutants are biochemically different. C141 has been placed in a third linkage group.

None of the mutants has been observed to accumulate any product in the medium which is biologically active for any of the other mutants. C84, C141 and T1710 accumulate biologically inactive compounds which appear to be imidazole derivatives. These compounds are different and those mutants can be differentiated by chromatographing culture fluids on filter paper.

All double histidineless mutants were isolated from intercrosses of the single mutants. Ability of the double mutants to accumulate those compounds accumulated by the single mutants and the histidine requirements of the double mutants were used to provide evidence for a proposed biosynthetic sequence corresponding to the single mutants.

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ISOCHROMOSOMES IN TOMATO

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CHROMOSOMES with two similar ends have been recognized since the pioneer work of BELLING and BLAKESLEE (1924) on the secondary trisomes of Datura. More recently, the term, isochromosome, has been applied by DARLINGTON (1939) to those chromosomes which have two identical arms. When found occurring naturally, the isochromosome has usually been shown to consist of inert, or B-type chromosomal material. Examples of such isochromosomes have been provided in rye by MÜNTZING (1944) and MÜNTZING and LIMA-DE-FARIA (1950), in sorghum by DARLINGTON and THOMAS (1941), in Anthoxanthum by ÖSTERGREN (1947), and in the moss *Dicranum majus* by VAARAMA (1950). On the other hand, in the isochromosomes of *Nicandra physaloides* studied by DARLINGTON and JANAKI-AMMAL (1945), the arms bear nucleolus-organizers and are apparently identical with an arm of the haploid complement.

Other examples of isochromosomes formed from members of the basic complement come from experimental cultures. They have usually been additions to the diploid complement, as in the secondary trisomes of Datura (BELLING and BLAKESLEE 1924), of maize (RHOADES 1933, 1940), and of tomato (LESLEY and LESLEY 1941). In cultures of wheat derived from pentaploid hybrids, LOVE (1940) discovered several examples of isochromosomes. In the diploid wheat species, *T. monococcum,* SMITH (1947) found an interesting situation in which an isochromosome from the long arm and a telocentric fragment from the short arm replaced one of the normal chromosomes.

In the genetically best studied example, the attached-X chromosome of *D. melanogaster,* the duplication originated from exchanges between the X and the two arms of the Y chromosome (NEUHAUS 1936; MORGAN 1938). Complexities in the study of heterochromatin have apparently prevented a detailed analysis of the similarities between the two arms of the attached-X in the region adjacent to the centromere or of the probable homologies between the two arms of the Y chromosome.

The present report is concerned with the cytological behavior of two different isochromosomes in the tomato and the transmission of one of them. As isochromosomes, they are unusual because each replaces a normal member of the diploid complement. The ability of the tomato to withstand such genetic unbalance is perhaps not unexpected since LESLEY and LESLEY (1941) have previously demonstrated a half-chromosome deficiency in the diploid complement. On the other hand, the transmission of such a half-chromosome

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deficiency to diploid progeny, as will be reported below, could not have been so readily anticipated.

Of further significance is the evidence on the behavior of the differentiated chromosomes of tomato in the heteromorphic state. Pachytene observations offer information on non-homologous chromosome association and also pose new problems in the interpretation of chromosome pairing.

ORIGIN OF THE ISOCHROMOSOMES

The two isochromosomes appeared in the F_1 mutants during an experiment on chemical mutagenesis in tomato. The material employed in these studies was a highly inbred line of the variety, Sutton's Best of All. Although X-rays and mustard gas produce genetic changes at a high rate in the tomato, neither of the mutagens used here produced such changes with sufficient frequency to be statistically significant in populations of the size studied. The two substances were formaldehyde and ammonia, used in vapor form to treat mature pollen. Additional observations in this laboratory tend to corroborate RICK's (1945) determination of the extremely low natural rate of genetic change in the tomato. On this basis it may be suggested that further studies will probably reveal a statistically significant effect of the weaker mutagens.

ISOCHROMOSOME 9L-9L

Morphology

Disome. When the isochromosome from the long arm replaced a normal chromosome nine in the diploid complement, the seedlings were dwarf and yellow-green. The first two leaves had mosaics of yellow patches on the upper surface and irregular purple streaks along the margin. The plant grew slowly and the stem was thin. The younger leaves had slightly yellowish humps on the upper surfaces along both sides of the midribs. In the older leaves, the leaflets drooped, and the terminal portion of the rachis curled downwards, giving the leaf its characteristic appearance (fig. 1). The styles were leafy and slightly twisted, and the fruits were small and infrequent. As the fruit grew, it developed five dark-green bands radiating along the surface from the stylar end. By means of sections these bands were shown to be parenchymatous tissue unusually full of chloroplasts. The bands disappeared as the fruit ripened.

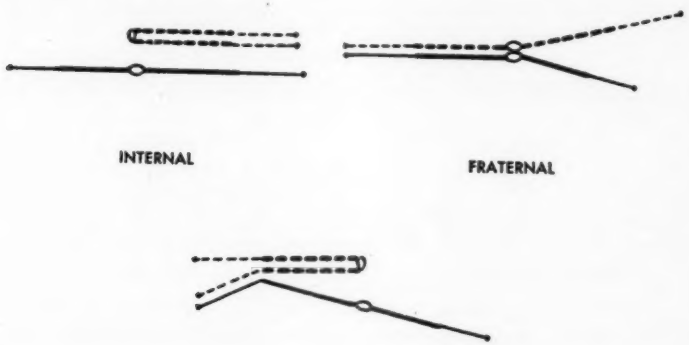
Trisomes. When the isochromosome 9L-9L was present in addition to the regular complement, the seedlings were dwarf but light-green rather than yellowish. In the greenhouse, the leaflets and rachis curled as in the diploid (fig. 2). In the field, however, the light-green color was the most noticeable variation while the leaflets tended to fold upwards instead of downwards. The fruits were small, and had the characteristic radiating green bands. Thus the secondary trisome was similar to the disome but less aberrant, particularly in the seedling stages.

The primary trisome for chromosome nine was derived from the secondary. The primary was intermediate in growth habit between the secondary and normal Sutton's Best. The leaves, however, did not show the curling character.

Cytology

The tomato chromosomes are characterized by chromatic zones adjacent to the centromere and distal achromatic regions which terminate in small, dark-stained telochromomeres (BROWN 1949). The boundary between these two zones is usually sharply defined, but occasional dark-stained chromomeres occur among the much smaller, lighter chromomeres of the achromatic zone. A chromosome can be identified on the basis of its total length, the relative length of its two arms, the relative lengths of the chromatic and achromatic zones in each arm, and its characteristic pattern of chromomeres. These characteristics have enabled BARTON (1950) to construct a complete map of the pachytene chromosomes. The cytological techniques employed in the present study were essentially those described by BARTON (1950).

Disome. At pachytene, three types of pairing were observed, and these are diagrammed in text fig. 1.

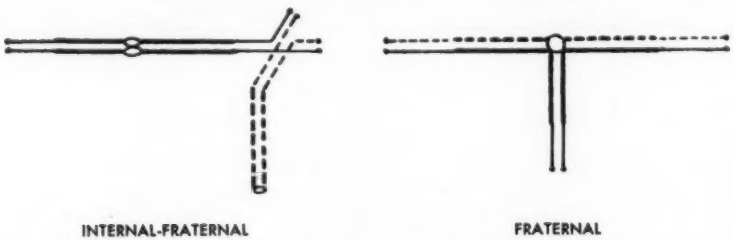


TEXT FIGURE 1.—Diagrams of the three types of pairing observed at pachytene in isochromosomal disomes. The normal chromosome is represented by a solid line, the isochromosome, by a broken line.

The two chromosomes were unpaired in the majority of the nuclei (figs. 5 and 7). In the isochromosome, the telochromomeres and the small dark-stained chromomeres of the achromatic zones seemed to be aligned in homologous association. In the chromatic zone, chromomere-by-chromomere pairing was difficult to study for the following reasons: (1) the chromatic chromomeres were packed densely together, (2) the pairing was comparatively loose, (3) the two arms frequently coiled around each other, obscuring the chromomere pattern, and (4) the two arms of the chromosome were often unequally stretched, displacing some of the chromomeres from their normal position. Identification of the isochromosome as a duplication of the long arm of chromosome nine (BARTON 1950) was based on the relative lengths of the chromatic and achromatic zones and the chromomere pattern. The morphology of the unpaired chromosome nine, and the very clear configurations in the secondary trisome served to corroborate this determination. The

internal-fraternal and the fraternal arrangements were observed less frequently than the internal. The normal chromosome nine was thus left to a large extent unassociated except for non-homologous pairing between sections of its own chromatic zones (figs. 7 and 8). The terminal fraternal association shown in figure 8 may be a union of the three telochromomeres rather than a 2-by-2 pairing of the normal with any part of the isochromosome.

One of the most striking features of the isochromosome at pachytene is the subterminal location of the centromere. Such a location might be attributable either to some type of non-homologous pairing or to a difference of one or two chromomeres between the two arms of the isochromosome; this problem will be considered further in the discussion. An examination of cells at late zygotene and early pachytene, where the chromatic zones were as yet unpaired, was considered likely to offer more information on the number of chromomeres (fig. 6). At this stage each of the chromatic segments appeared to have ten dark-stained chromomeres of corresponding sizes yet the unequal stretching of the interchromomeric material still left some room for doubt.



TEXT FIGURE 2.—Two of the types of configuration observed at pachytene in the secondary trisome. The normal chromosomes are represented by solid lines, the isochromosome, by a broken line.

At diakinesis, the two chromosomes were unassociated in about half the cells. The normal chromosome nine was a rod-shaped univalent while the isochromosome was usually ring shaped because of a chiasma in the achromatic zone. These configurations were very similar to those of the other isochromosome (fig. 4). At metaphase, the larger univalent was assumed to be the isochromosome on the basis of sizes at pachytene and diakinesis. This assumption was later confirmed by the presence of one large and two small chromosomes in the secondary trisome configuration. At metaphase, the univalents were frequently observed between the poles and the remainder of the complement at the equatorial plate. Sometimes both members of the heteromorphic pair went to one pole (fig. 3), but more commonly to opposite poles. Univalents occasionally divided at first anaphase, but lagging without division seemed more common. This behavior gave rise, as might be expected, to some quartets with 11- and 13-chromosome nuclei. Thus there was ample reason for expecting the heterozygous disome to give rise to secondary trisomes.

About fifty percent of the pollen produced by the disome was empty.

TABLE 1

Frequency of the different types of configurations at diakinesis in a secondary and a primary trisomic tomato.

Trisomic type	Configuration								Total
	— =	o =	/	○	∩	Y	∩	Y	
Secondary 9L-9L	3	16	16	9	2	1	2	1	50
Primary 9L-9S	19	....	18	....	....	....	9	4	50

Trisomes. In the secondary trisome, the isochromosome was present in addition to the regular diploid complement. In pairing, it might be expected to yield either a complete association with the two normal chromosomes, or to be partially internally paired (text fig. 2). Both types of configurations were found, but the complete associations were analyzed with greater surety (figs. 9 and 10). Such complete associations might be taken for evidence that the two arms of the isochromosome are absolutely identical. A possible objection to this assumption will be considered in the discussion.

The typical trivalent configurations expected in a secondary trisome were observed at diakinesis (figs. 11–15). As may be seen from table 1, over half of the configurations found in the secondary are of those types which can not occur in a primary trisome.

About thirty percent of the pollen produced by the secondary trisome was empty while the primary yielded almost as much abortion, or twenty-five percent.

Transmission

The crosses made with the isochromosomal disome are summarized in table 2. The data are not inconsistent with the conclusion that the isochromosome was transmitted almost exclusively by the female parent. The disome reproduced its own condition at a rate of less than one percent, or approximately six per thousand. It produced secondary trisomes at a rate almost ten times greater, or approximately five per hundred. Because the frequency of gametes with a deficient chromosome was obviously much greater than that of $n+1$ gametes, this difference must have been due solely to differences in functioning or viability in the gametes or young zygotes.

TABLE 2

F_1 progenies of the reciprocal crosses and of the selfed curly-leaf mutant.

Parents	No. of seeds sown	No. of seeds germ.	No. of curly-leaf trisomes	No. of curly-leaf disomes
Curly-leaf disome × tester stock	48	45	3	....
Tester stock × curly-leaf disome	43	43	....	....
Sutton's Best × curly-leaf disome	192	180	....	....
Curly-leaf disome × Sutton's Best	48	44	1	....
Curly-leaf disome selfed	532	503	25	3

In 107 seedlings grown from selfing the secondary trisome, twenty proved to be secondaries while two were primaries. Thus the secondary was about four times as effective as the disome in the production of secondary trisomes.

ISOCHROMOSOME 8L-8L

The isochromosome composed of two long arms from chromosome 8 was studied only morphologically and cytologically. The fertility of this isochromosomal disome was so low that studies of transmission have not yet been possible. Its occurrence, however, demonstrates that chromosomes other than nine can be replaced by isochromosomes in the diploid complement.

In morphology, this isochromosomal disome was not distinguishable in the seedling stage. The plant grew slowly, the leaves were dark-green in color, small in size, and had narrow leaflets with fewer and smaller hairs. The inflorescences were occasionally branched.

At pachytene, the behavior was similar to that described above for isochromosome 9L-9L. The centromere appeared subterminal in some instances, and terminal in others (fig. 16). Pairing with the normal chromosome 8 was usually restricted to the long achromatic region while the normal chromosome itself exhibited non-homologous association in its chromatic zones.

At diakinesis, the two chromosomes were present as a bivalent in about fifty percent of the cells. As univalents, one was rod-, the other usually ring-shaped (fig. 4). Behavior during later phases was also similar to that seen in the other disome. Pollen fertility was considerably lower than in the other disome, with seventy as compared to fifty percent of the grains aborted. Fruit set was almost non-existent as only one fruit was obtained from many trials of natural or artificial selfing.

DISCUSSION

Transmission. In general in plants, the longer the deficiency, the less frequently it is transmitted through the gametophytic generation, the male gametophyte usually being much less tolerant than the female. The diploid plant is able to tolerate deficiencies which it is unable to transmit except at markedly reduced rates. In addition, the genetic unbalance caused by the deficiency may have specific effects rendering the diploid infertile. Thus there are no reports of transmission of the monosomics observed in *Nicotiana alata* var. *grandiflora* (AVERY 1929), in maize (MCCLINTOCK 1933), or in Petunia (RICK 1943). If a deficient chromosome is unpaired with its normal homologue, it may be transmitted much more frequently as an addition to the regular complement. Such behavior for half-chromosome fragments has been recognized in *T. monococcum* by SMITH (1947) and in tomato by LESLEY and LESLEY (1941).

The results reported here fall in line with these other records. The isochromosome 9L-9L is transmitted very poorly as a substitute for a normal chromosome 9, but much more readily as an addition to the regular complement. In his studies of a long haplo-viable deficiency in maize, STADLER (1933) pointed out that factors affecting gametophyte development may not

necessarily be distributed at random along the chromosomes. Even though the deficiency for an entire arm is a longer transmissible deficiency than any previously reported, it would seem better to judge the genetic importance of the lost material on the fact that the deficiency is transmitted rather than on the chromosome length involved.

The secondary trisome from isochromosome 9L-9L reproduced itself at a rate similar to that of the several primaries reported for tomato (LESLEY 1928), and intermediate for those reported for the secondaries of Datura (BLAKESLEE and AVERY 1938). Likewise, the secondary gave rise to the related primary at a rate within the range found for the secondaries in Datura.

Cytological behavior. Previous work with the tomato chromosomes has shown that the distal achromatic zones and the centromeres usually pair prior to the proximal chromatic zones (BROWN 1949). The paired centromeres tend to adhere to other paired centromeres. In translocation-heterozygote configurations at pachytene, the centromeres brought into proximity may adhere very frequently, thus inducing non-homologous association in the adjacent chromatic zones (BARTON 1949). The isochromosomal materials offer a further basis for comparing the behavior of the different chromosome regions.

At the diploid level, internal pairing of the isochromosomes was much more frequent than either the internal-fraternal or the fraternal types. A similar situation seems to have prevailed in an isochromosome in *T. monococcum* (SMITH 1947) and in a duplication in wheat (FRANKEL 1949). On the trisomic level, where four homologous arms are present, the isochromosome much more often paired completely with the homologous arms of the normal chromosome. On the disomic level, the centromeres of the normal and isochromosome did not, as a rule pair with each other. Perhaps they must first be brought into propinquity by pairing of the distal achromatic zones, as in normal bivalents.

Non-homologous association. Non-homologous behavior was confined to the chromatic zones while the normally more active achromatic regions remained unassociated unless homologously paired. In her studies of non-homologous association in maize, MCCLINTOCK (1933) concluded that the non-homologous fold-backs originated near the centromere because the telophase orientation of the chromosome resulted in these regions being the closest together. In the non-homologous associations observed in the tomato, the fold-backs could occur anywhere within the chromatic zone. Also, the telochromomeres were sometimes stuck to the chromatic zones while the achromatic material in between formed an open loop. Although not differentiated in a striking fashion, maize chromosomes usually seem somewhat more chromatic near the centromere. Likewise, nearly all of the non-homologous associations of the telocentric B-chromosome had their origins in the heavily chromatic mid-region of this chromosome.

Although not a non-homologous association, union in a group of three is certainly different from normal 2-by-2 pairing. MCCLINTOCK (1933) reported such triple unions for terminal knobs in maize, and OLMO (1934) found triple unions in triploid *Nicotiana tabacum* to be much more frequent

for the chromatic chromomeres near the centromere and the terminal chromomeres than elsewhere along the chromosome. LONGLEY (1937) has pictured striking examples of multiple fusion of knobs in teosinte. The adhesions of heterochromatin are well known for Drosophila and have also been reported for other animals. Thus the chromaticity of the chromosome region may be a factor of general importance in non-homologous association although, perhaps, be clearly apparent only in those cases in which the chromosomes are conspicuously differentiated.

Pairing of the isochromosome in relation to its origin. The least understandable feature of the pachytene configurations was the subterminal location of the centromeres. Obviously, exact pairing between two identical arms should have produced a fold-back in the centromere itself. In isochromosome 9L-9L, the centromere was very infrequently terminal while in 8L-8L it was terminal in only about 50 percent of the configurations. Three different explanations may be suggested. (1) One arm may differ from the other by the presence of two or three extra chromomeres; the centromere and these extra chromomeres would then pair non-homologously. (2) The centromere may pair non-homologously with adjacent chromomeres with sufficient strength to overcome the homologous association in the chromatic zone. (3) The centromere may be sufficiently resistant to bending to force the adjacent chromatic zone to undergo bending and to pair non-homologously.

The first two explanations seem highly unlikely. If one arm of the isochromosome contained extra chromomeres, then it could have been formed only by a minimum of two breaks. In view of the very low frequency of breakage in the control and the treated series, the simultaneous occurrence of two breaks in a single chromosome on two different occasions seems very improbable. Furthermore, the fact that the chromatic zones and the centromere form a linear structure in the resting nucleus makes a union between the two arms of the same chromosome seem quite unexpected. Unfortunately, the very clear configurations of the secondary trisome can not be used for evidence here because of the tendency for adjacent centromeres in such configurations to stick together (BARTON 1949). The second explanation must also be ruled out because the centromere of the normal chromosome does not exhibit a tendency toward non-homologous association. For the third explanation, that of resistance of the centromere to mechanical distortion, there is no independent evidence. The relatively much thicker centromere might, however, be expected to be less pliable than the very delicate strands running between the heavy chromomeres of the chromatic zone.

Origin. In the preceding paragraphs, reasons have been given for assuming that the isochromosomes studied here arose as a result of breakage at the centromere. Following DARLINGTON's (1939, 1940) observations, isochromosomes might be expected to arise from univalent chromosomes lagging at meiosis. Similarly, misdivision of telocentric fragments may give isochromosomes (RHOADES 1940; LESLEY and LESLEY 1941). DARLINGTON and KOLLER (1947) reported that nitrogen mustard, among its other effects, induced many disturbances at the centromere. In the present case, the evi-

dence for the chemical induction of the isochromosomes is not statistically significant. It seems more likely, however, that the isochromosomes arose as a result of treatment rather than as spontaneous aberrations.

SUMMARY

Two cases are reported in which an isochromosome replaced a member of the diploid complement in tomato. The genetic unbalance produced characteristic morphological changes.

In one case, infertility prevented studies of transmission. In the other, the isochromosome was transmitted by the female parent. In the progeny, it appeared as a disomic at the rate of six per thousand, as a secondary trisomic, of five per hundred. The secondary trisomic produced about twenty percent secondaries and two percent primaries.

In later meiotic stages, the isochromosomes behaved typically, forming ring univalents, heteromorphic bivalents, and ring trivalents, among other configurations. The isochromosomes were identifiable at pachytene on the basis of BARTON's map. Internal pairing of the isochromosome was much more frequent than fraternal or internal-fraternal pairing. In the normal homologue of the isochromosome, non-homologous association was confined to the chromatic zone. The relationship of this observation to other examples of non-homologous association is considered in the discussion.

At pachytene, the centromeres of the internally paired isochromosomes are very frequently subterminal. Reasons are given for assuming that this position is not attributable either to a slight difference in homology between the arms or to non-homologous association. The explanation is offered that the centromere may possess resistance to mechanical distortion.

ACKNOWLEDGMENTS

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PLATES I AND II

(Photomicrographs enlarged 4000 ×; in the companion diagrams the normal chromosomes are represented by solid, the isochromosomes, by broken lines.)

PLATE I

FIGURE 1.—Leaves of the curly-leaf mutant.

FIGURE 2.—Curly-leaf secondary trisome (greenhouse culture).

FIGURE 3.—Precocious movement at first anaphase of the larger isochromosome 9L-9L and smaller chromosome 9.

FIGURE 4.—Univalents at diakinesis: chromosome 8 is the upper rod, isochromosome 8L-8L is the lower ring.

FIGURE 5A & B.—Isochromosome 9L-9L internally paired at pachytene; note subterminal position of centromere.

FIGURE 6A & B.—Isochromosome 9L-9L at late zygotene; the chromatic segments of the two arms form a loop, the achromatic segments have already paired.

FIGURE 7A & B.—Isochromosome 9L-9L internally paired at pachytene: chromosome 9 shows non-homologous association in the tips of the chromatic region; note subterminal position of centromere of the isochromosome.

PLATE II

FIGURE 8A & B.—Isochromosome 9L-9L and chromosome 9 at pachytene: chromosome 9 shows non-homologous association in most of its chromatic zone; see text for discussion of triple union at telochromomere.

FIGURE 9A & B; 10 A & B.—Complete association of isochromosome 9L-9L with two chromosomes 9 in pachytene of secondary trisome.

FIGURE 11-15.—Trivalents at diakinesis of the secondary trisome of isochromosome 9L-9L.

FIGURE 16.—Isochromosome 8L-8L internally paired at pachytene; note terminal position of centromere.

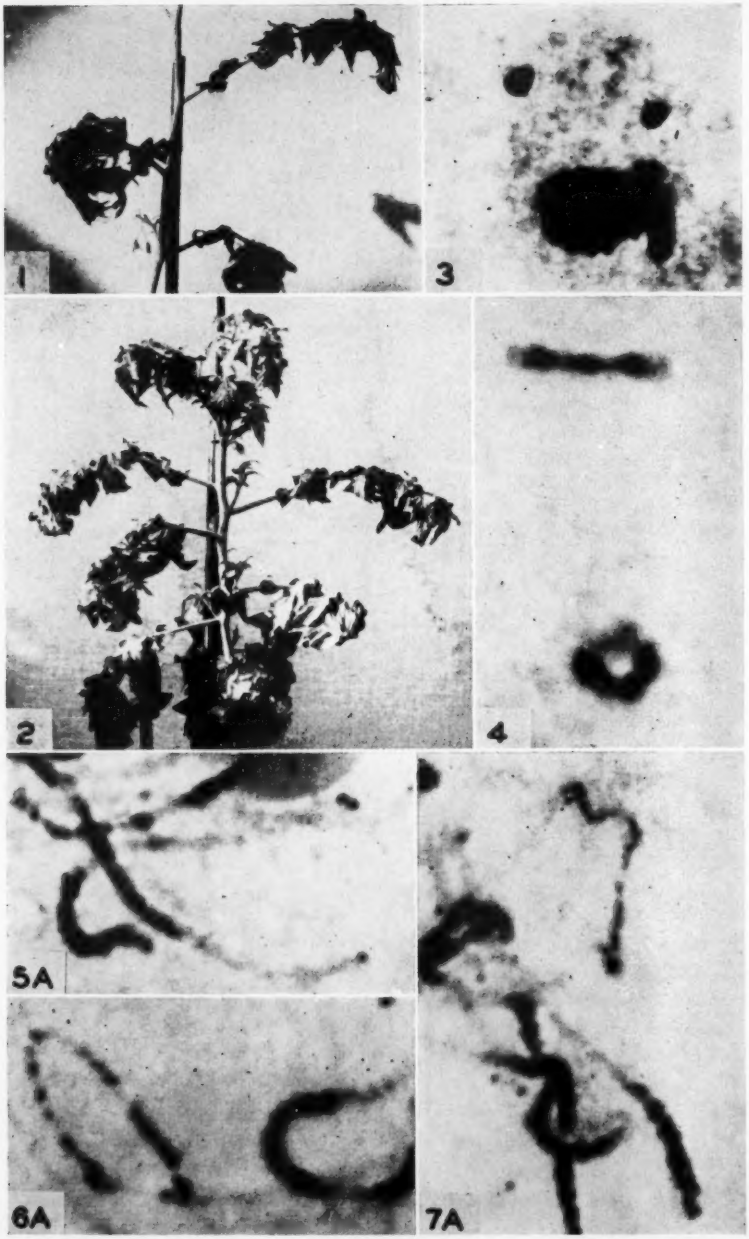


PLATE I

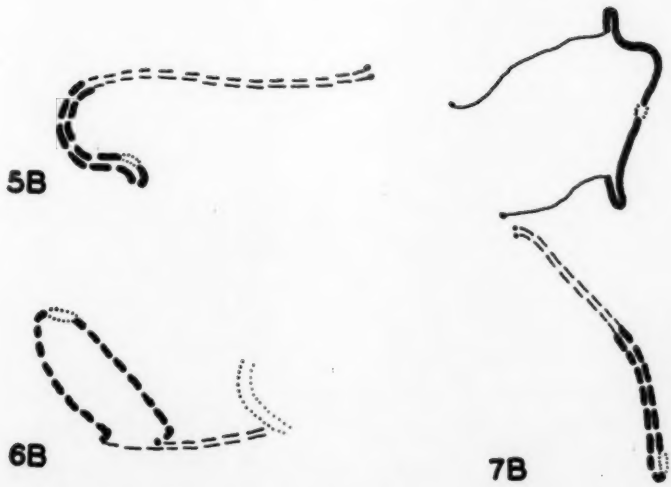


PLATE I

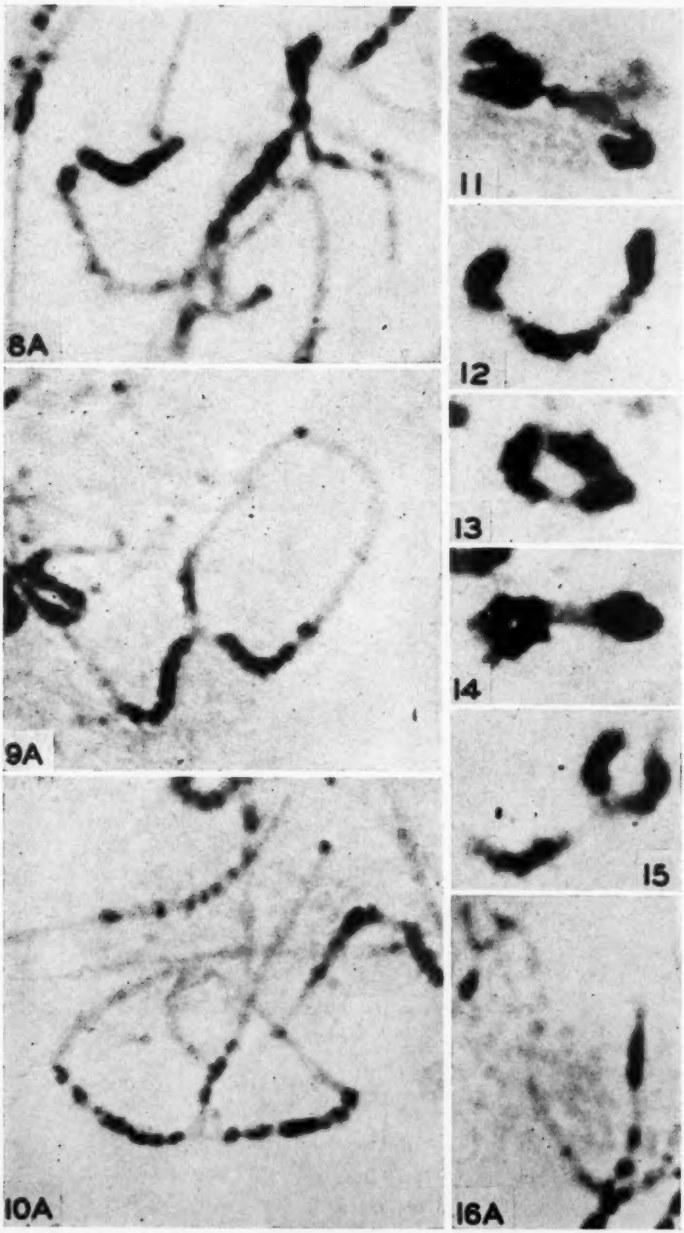
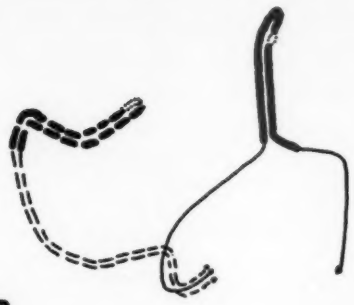
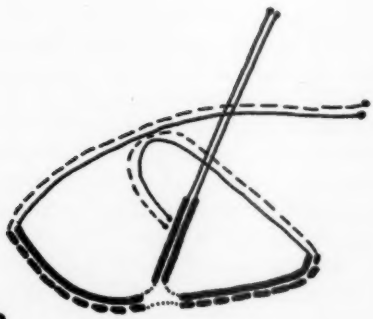


PLATE II



8B

9B



10B

16B

PLATE II

POLYGENOMIC HYBRIDS IN GOSSYPIUM. I. CYTOLOGY OF HEXAPLOIDS, PENTAPLOIDS AND HEXAPLOID COMBINATIONS[1]

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THE genus Gossypium is in many respects an unusually favorable group for the study of species relations. Cytologically, the genus comprises two groups of species, diploids with $n = 13$, and a group of three amphiploids of related origin, combining two separate genome groups, with $n = 26$. *G. barbadense* and *G. hirsutum*, the New World cultivated cottons, are members of the latter group. Of the six cytologically differentiated genome groups defined by BEASLEY (1940), A, B, C, D, E and (AD), each will cross with a representative of at least one other genome. Certain of these crossing relationships have recently been reviewed by BROWN and MENZEL (1952). However, attempts to obtain hybrids between certain species by direct crossing have failed; hence their cytological homology has been studied only indirectly by comparing the behavior of each in hybrids with a third species which is compatible with both.

It has been possible in many instances to combine in one plant two species which will not cross directly, as well as those which will, by crossing one species with an amphiploid involving the second. Two series of amphiploids, tetraploids from doubled F_1 hybrids between diploid species, and hexaploids from doubled hybrids of natural allotetraploids × diploid species, have been produced at the Texas Agricultural Experiment Station (many of them by J. O. BEASLEY prior to 1942). These synthetic amphiploids may be crossed among themselves and with natural species, both diploid and tetraploid. Such crosses have augmented the genome combinations possible by direct species crosses. It has been possible so far in Gossypium to obtain plants containing complete chromosome sets from two, three or four species, thus forming series with the diploid, triploid, tetraploid, pentaploid or hexaploid multiples of the basic chromosome number. One such series of hybrids, crosses of hexaploids with diploid species, has been reported previously (BROWN and MENZEL 1950). A number of additional series involving all obtainable combinations of natural species, synthetic allotetraploids (BROWN 1949) and synthetic allohexaploids are being made and studied cytologically at this laboratory. When these studies are completed, it is expected that they will yield information on the operation of barriers to species crossing and gene interchange in Gos-

[1] Contribution from Department of Agronomy, Cotton Investigations Section, TEXAS AGRICULTURAL EXPERIMENT STATION, College Station, Texas, as Technical Article No. 1504. A part of the work was done under Project S-1 of the Research and Marketing Act of 1946.

sypium, the extent and nature of cytological differentiation among and within genome groups, and perhaps some further clues to the phylogenetic history of the genus.

Moreover, such complex hybrids are of some ultimate practical interest in the long range project of transferring into *G. hirsutum* desirable characters which are outside the present range of variability of the cultivated cottons. To this end, characters from species with chromosomes which form a high number of chiasmata with those of *G. hirsutum* are most likely to be transferred readily. However, since there is a considerable range of chromosome homology among species of Gossypium, it may also prove possible to transfer some characters into *G. hirsutum* from genomes having very little homology with either of the *hirsutum* subgenomes. One possible approach, which will be discussed in a later paper, is through the addition of whole chromosomes and chromosome pairs. A second approach is theoretically possible, namely, first introducing characters from one species by crossing over into chromosomes of another diploid species having greater homology with *hirsutum* chromosomes, and thence introducing them into *G. hirsutum*. It may reasonably be expected that a study of pairing in complex hybrids involving three or more species will be useful in delimiting the maximum possibilities of such a double transfer technique.

The present paper deals with the cytology of a series of trispecies hybrids obtained from intercrossing allohexaploids, each of which was a doubled hybrid of the natural allotetraploid *G. hirsutum* and a different diploid species. The hybrids therefore contain 78 chromosomes, 52 (4 complete subgenomes) from *G. hirsutum* and 13 (one genome) from *each* of two diploid species. The hexaploids themselves, and the pentaploids resulting from crossing them with *G. hirsutum*, are also discussed for purposes of comparison.

Genome symbols used in this paper, after BEASLEY (1940, 1942) and BROWN and MENZEL (1950), are as follows:

G. herbaceum	A_1	*G. klotzschianum*	D_3
G. arboreum	A_2	*G. aridum*	D_4
G. anomalum	B_1	*G. raimondii*	D_5
G. sturtii	C_1	*G. stocksii*	E_1
G. thurberi	D_1	*G. hirsutum*	$(AD)_1$
G. armourianum	$D_{2\text{-}1}$	*G. barbadense*	$(AD)_2$
G. harknessii	$D_{2\text{-}2}$		

Subgenomes of *G. hirsutum*, when referred to separately, are designated A_h and D_h. A capital letter without a subscript refers collectively to all species of that genome group. Symbols M and N are used to designate any two different diploid genomes. For brevity, " diploid genome " will refer to one 13-chromosome complement from a diploid species. Since all the present amphiploids contain *G. hirsutum*, it will often be convenient to refer to them by the diploid species they contain; *e.g.*, the hexaploid $2(AD)_1C_1$ will be called the "*sturtii*" or "C_1" hexaploid. The genome formulae of the hexaploid × hexaploid hybrids

will be written (AD)M/(AD)N, the first term representing the genomes contributed by the seed parent.

A set of tables showing complete cytological data for each hexaploid × hexaploid hybrid is being deposited with the EDITORS OF GENETICS, for distribution upon request.[2] Hence only average pairing and pertinent details will be given here.

HEXAPLOIDS

The following hexaploids were used in these studies:

doubled *hirsutum–herbaceum*	$2(AD)_1A_1$
doubled *hirsutum–arboreum*	$2(AD)_1A_2$
doubled *hirsutum–anomalum*	$2(AD)_1B_1$
doubled *hirsutum–sturtii*	$2(AD)_1C_1$
doubled *hirsutum–thurberi*	$2(AD)_1D_1$
doubled *hirsutum–armourianum*	$2(AD)_1D_{2\text{-}1}$
doubled *hirsutum–harknessii*	$2(AD)_1D_{2\text{-}2}$
doubled *hirsutum–raimondii*	$2(AD)_1D_5$
doubled *hirsutum–stocksii*	$2(AD)_1E_1$

All were produced by colchicine doubling of F_1 hybrids except two of the five *armourianum* hexaploids which arose by spontaneous doubling (BROWN 1951). In most cases, the original colchicine-doubled plants were maintained by grafts of fertile branches. Most hybrids involving the *raimondii* hexaploid were derived from hexaploid seedlings of the original amphiploids. Since little segregation was noted in these seedlings, they are here considered cytologically equivalent to the original doubled parent. The *harknessii* hexaploids were also seedlings, which were grown from seeds kindly supplied by S. G. STEPHENS from one of his stocks.

The morphology of the hexaploids will be discussed in detail in a later paper, in connection with other amphiploids and their derivatives. In general, the hexaploids, like the F_1 hybrids, are intermediate between *G. hirsutum* and the diploid parent. Influence of the wild genome on individual plant parts varies markedly from one species to another, but both the diploid and the *hirsutum* components are clearly discernible in all. The amphiploids are generally distinguished from their corresponding F_1 hybrids by larger, thicker, less deeply lobed leaves, larger and broader bracteole and flower parts, larger anthers and more regular shedding of pollen, and often by more irregular, ruffled, somewhat lobed petal margins. Similar observations have been made by HARLAND (1940), AMIN (1941) and BROWN (1951). The occurrence of color mosaics in petals and leaves of the original hexaploids, their seedlings and hybrids, and in a number of other types of polygenomic hybrids, is discussed in the second paper of this series (MENZEL and BROWN, 1952).

[2] To obtain supplementary tables, order Document No. 3491 from the AMERICAN DOCUMENTATION INSTITUTE, 1719 N St., N.W., Washington 6, D. C., remitting $1.00 for microfilm or $4.05 for photocopies.

Fertility

The triploid hybrids from which the hexaploids are derived are almost completely sterile. All of the hexaploids, on the other hand, are more or less fertile. The degree of fertility varies considerably with the diploid species involved. The E_1 hexaploids are most prolific, readily setting many selfed bolls with a high number of seeds. The A_1, A_2, B_1 and D_1 hexaploids are also rather highly self-fertile, while the D_5 hexaploids self only occasionally, and the $D_{2\text{-}1}$ (both spontaneous and colchicine-induced) very rarely set selfed seeds even when hand pollinated (BROWN 1951). A hexaploid ***barbadense–raimondii*** produced by STEPHENS (1942) proved highly sterile, but it was somewhat male-fertile when crossed with *G. barbadense*. HARLAND (1940), however, reports that a hexaploid ***barbadense–armourianum*** was fully male- and female-fertile, as was a hexaploid ***barbadense–aridum***, while a ***barbadense–thurberi*** hexaploid was male-sterile but fully female-fertile with ***hirsutum*** or ***barbadense*** pollen. AMIN (1940, 1941) and BEASLEY (1942) also found hexaploids of ***barbadense*** and ***hirsutum*** with ***herbaceum*** to be partially to fully fertile.

Regardless of the degree of self-fertility, all of the above hexaploids are at least partially fertile as male or female parent, or (usually) both, when crossed with *G. **hirsutum*** or with a hexaploid involving a different diploid species.

The C_1 hexaploid deserves special consideration in this respect. Like the $D_{2\text{-}1}$ and D_5 hexaploids, it rarely sets bolls, either when selfed or when outcrossed. Crosses, however, are readily obtained using the C_1 hexaploid as the male parent. This hexaploid suffers from a peculiar somatic incompatibility, and although long-lived, continually dies back at the tips of branches and produces distorted, cupped, small, mottled leaves. Some nodes appear to develop only enlarged stipules, and there is considerable shortening of the internodes. A similar but less marked distortion and general unthriftiness characterize some hybrids of which it is either male or female parent, so that it seems unlikely that the derangement is due entirely to a residual effect of colchicine treatment. The triploid $(AD)_1C_1$ from which the hexaploid is derived has rather small cupped leaves, but the growing tips are normal, as are those of the pentaploid $(2(AD)_1)C_1$ obtained from crossing the hexaploid with *G. **hirsutum***. The flower of both F_1 and hexaploid are quite unaffected by the distortion. It seems possible that the low female fertility of the C_1 hexaploid is due to a general unfavorable physiological condition of the whole plant, rather than to specific factors affecting reproduction only, in contrast to the probable situation in the $D_{2\text{-}1}$ and D_5 hexaploids.

Chromosome pairing

IYENGAR (1944) and BROWN (1951) have published detailed analyses of several hexaploids, and their results, together with the average pairing found in the plants in the present study, are summarized in table 1. AMIN (1941) reports a typical association of 5 I, 18 II, 3 III and 7 IV in a pollen mother cell of a fertile branch of colchicine-treated ***hirsutum–herbaceum***. One cell

TABLE 1

Chromosome pairing in pollen mother cells of allohexaploids.

Genome formula	Average							No. plates	Authority	Maximum IV
	II	III	IV	V	VI	VIII	I			
$2(AD)_1A_1$	29.50	0.88	3.58		0.13		1.29	24	Iyengar 1944	7
$2(AD)_2A_1$	34.63	1.38	0.63				2.13	16	Iyengar 1944*	3
$2(AD)_1A_1$	25.62	1.16	5.19	0.06	0.19		0.97	32	authors	10
$2(AD)_1A_2$	28.50	1.13	2.38	0.13	1.00		1.50	8	Iyengar 1944	5
$2(AD)_1A_2$	27.50	0.80	4.42	0.04	0.15	0.04	1.27	26	authors	10
$2(AD)_2B_1$	36.50	0.30	0.50			0.05	1.70	20	Iyengar 1944	2
$2(AD)_1B_1$	35.43	0.50	0.60	0.07			2.90	30	Iyengar 1944	2
$2(AD)_1B_1$	35.71	0.58	0.71		0.11		1.34	51	authors	3
$2(AD)_1C_1$	36.54	0.32	0.57				1.68	28	authors	2
$2(AD)_1D_1$	28.72	0.59	4.40	0.04			0.90	22	authors	10
$2(AD)_2D_1$	33.96	0.42	2.00		0.04		0.67	24	Iyengar 1944	6
$2(AD)_2D_{2-1}$	32.75	0.86	1.75	0.02	0.04		2.32	28	Iyengar 1944	5
$2(AD)_1D_{2-1}$	25.81	1.18	5.19				1.26	68	Brown 1951	12
$2(AD)_1D_{2-2}$	29.09	1.03	3.54		0.03		2.16	31	authors	7
$2(AD)_1D_5$	27.93	0.31	4.34				0.93	29	authors	9
$2(AD)_1E_1$	38.06	0.13	0.06				1.16	31	authors	1

*Computed average from IYENGAR'S data for two plants.

showed 28 II, 1 III, 2 IV, 1 V and 1 VI. Hexaploids of *barbadense–herbaceum* showed similar pairing. BEASLEY (1942) also studied pairing in four hexaploids, $2(AD)_1A_1$, $2(AD)_1B_1$, $2(AD)_1C_1$ and $2(AD)_1D_{2-2}$. He found associations of four to be much lower in $2(AD)_1A_1$ than expected if the A_1 genome were identical with A_h. Five or more structural differences were postulated between D_{2-2} and D_h, and the A_1 hexaploid was more fertile than the D_{2-2}. In $2(AD)_1B_1$, 50 percent of the cells had 39 II's; the commonest irregularities were I's, and rarely multivalents were formed. The C_1 hexaploid usually showed one or more associations of four chromosomes and two or more univalents.

The present data on metaphase pairing agree fairly well with previous reports. The widest discrepancies are found in hexaploids involving the D genomes, probably because counts of II's and IV's are open to greater subjective error in these, where chromosomes of four of the six genomes are quite small, than in those incorporating the larger A, B, C or E genomes. As pointed out by BROWN (1951), it is often difficult to decide in the D hexaploids whether four closely associated chromosomes are paired as two II's or as one IV of the figure-8 type. The average number of III's, I's and multivalents agrees rather well from one report to another, and the discrepancy of II–IV

frequency is of only slight significance in comparing chromosome conjugation in the hybrids between hexaploids (see below).

G. hirsutum has been amply demonstrated to be an allotetraploid comprised of two A and two D genomes (SKOVSTED 1937; HARLAND 1940; BEASLEY 1940, 1942; BOZA BARDUCCI and MADOO 1941; BROWN 1951; BROWN and MENZEL 1950). Pairing in hybrids of the constitution (AD)D has recently been reviewed by BROWN and MENZEL (1952). All show approximately 13 small (D) II's and 13 larger (A) I's. Pairing in (AD)A hybrids, while complicated by multivalent formation, nevertheless conforms closely to the expected 26 large paired chromosomes and 13 smaller I's (NAKATOMI 1931; SKOVSTED 1934; FENG 1935; WEBBER 1939; AMIN 1941; BEASLEY 1942; IYENGAR 1944, and unpublished data of the authors).

Hence the maximum number of associations of four or more chromosomes expected in either 2(AD)A or 2(AD)D hexaploids is 13. Inspection of table 1 shows that the average multivalent formation in all A and D hexaploids is far below the maximum, and that it is rarely if ever realized even in individual plates. This may be due partly to selective pairing, indicating differentiation which undoubtedly exists between A and A_h, and D and D_h genomes, as BEASLEY concluded for the A_1 hexaploid. It is probably a function also of the low chiasma frequency in the genus (see STEPHENS 1950), since chiasmata per metaphase chromosome rarely exceed two, and since the expected maximum of 26 IV's in doubled *G. hirsutum* is likewise seldom realized (MENDES 1940; BEASLEY 1942; BROWN 1947).

There is less general agreement as to the maximum pairing possible in triploids of *G. hirsutum* with the three genome groups not ancestral to it.

TABLE 2

Comparison of bivalent formation in triploid hybrids involving the B, C and E genomes with quadrivalent formation in their corresponding hexaploids.

Triploids			Corresponding hexaploids		
Genome formulae	Authority	Average II	Average IV	Maximum IV	Authority
$(AD)B_1$	Webber 1936	usually 13			
$(AD)_1B_1$	Webber 1939	10.48	0.58	2	authors
$(AD)_2B_1$	Skovsted 1937	2.6			
$(AD)_2B_1$	Beasley 1942	8.9			
$(AD)_2B_1$	Iyengar 1944	6.4	0.30	2	Iyengar 1944
$(AD)_1B_1$	Iyengar 1944	5.2	0.50	2	Iyengar 1944
$(AD)_2C_1$	Webber 1935	0.68			
$(AD)_2C_1$	Skovsted 1937	3.1			
$(AD)_2C_1$	Skovsted 1937	5.25			
$(AD)_1C_1$	Skovsted 1937	5.65	0.57	2	authors
$(AD)_1C_1$	Skovsted 1937	6.35			
$(AD)_1C_1$	Skovsted 1937	7.6			
$(AD)_1C_1$	Webber 1939	0.52			
$(AD)_2E_1$	Skovsted 1937	0.55			
$(AD)_1E_1$	Beasley 1942	(1.9 bridges)	0.06	1	authors

Table 2 compares the average number of II's reported by various authors for (AD)B, (AD)C and (AD)E hybrids with the average and maximum number of IV's in the corresponding hexaploids. Despite the range of pairing reported for the triploids, especially for $(AD)B_1$, it is obvious that IV formation in the B, C and E hexaploids is also very much lower than the theoretical maximum. The deficiency is particularly striking in the $2(AD)B_1$ hexaploid, since B_1 apparently can form a maximum of 13 II's with (AD), and pairs regularly enough with A_1 and A_2 to give a partially fertile F_1 (SILOW 1941).

The $2(AD)_1E_1$ hexaploid has apparently never previously been analyzed cytologically. BEASLEY (1942) considered it doubtful that any pairing occurred between (AD) and E_1 since pairing in the triploid did not exceed that in haploid *G. hirsutum*. Of 31 pollen mother cells analyzed, however, two had 1 III and one had 1 IV. Multivalents are very rarely seen in *G. hirsutum* itself, so that it is probable that at least one E_1 chromosome may form a chiasma with a pair from *G. hirsutum*. This point is confirmed by the discovery of a plant with 55 chromosomes in a progeny of an E_1 pentaploid (hexaploid × *G. hirsutum*) backcrossed to *G. hirsutum*. This plant usually had 26 II + 3 very large I's at metaphase I, but occasionally a configuration of 25 II, 1 III and 2 I was observed.

STEPHENS (1945) discussed the somewhat anomalous relationships of *G. stocksii* to the other Old World species: Evidence from comparative morphology, geographic distribution and crossing behavior seems to indicate a closer affinity to *G. herbaceum*, *G. arboreum* and *G. anomalum* (and hence to the A_h subgenome) than the cytological behavior warrants. He suggests that specific genic control of pairing may be implicated. Such genes are known in Gossypium (BEASLEY and BROWN 1942), but doubling of asynaptic plants fails to increase pairing, while doubling of the $(AD)E_1$ hybrid, as well as of A_2E_1 (BEASLEY 1942), restores both nearly perfect pairing and high fertility. Hence a genetically simple mechanism reducing pairing in E_1 hybrids seems to be precluded.

STEPHENS (1942) also predicted that amphiploids of New World cottons and either *G. sturtii* or *G. stocksii* would prove the only possibility of obtaining fully fertile cotton species with more than 52 chromosomes. The prediction is confirmed in the *stocksii* hexaploid. It not only exhibits the most regular pairing of all the hexaploids, but is exceedingly fertile: indeed, it is perhaps more prolific under local greenhouse conditions than commercial strains of *G. hirsutum*.

The above comparison of chromosome pairing in F_1 species hybrids with that in the corresponding amphiploids is of value to the ultimate objective of transferring putative "useful" characters from diploid species into *G. hirsutum*. Pairing in the F_1 hybrids, in which each genome is present only once, provides an index to the absolute maximum chiasma (= crossover) frequency possible between differentiated genomes. The amphiploids, in which two or more genomes are present twice, give an estimate of the frequency of such chiasmata likely to be realized in derived lines fertile enough for breeding and selection experiments. In all of the allohexaploids, the frequency of inter-

genomic pairing is very much lower than in the corresponding F_1 hybrids. It is virtually negligible in allohexaploids involving the B, C and E genomes, and in the following discussion of pairing in pentaploids and hexaploid combinations, it will be considered that gametes from B, C and E hexaploid parents had practically perfect (AD)N genome constitution. In the A and D hexaploids the frequency of substituted chromosome segments appears to be appreciably higher. However, since pairing in F_1 hybrids (AD)A and (AD)D is practically complete, indicating very high homology between A_h and A, and between D_h and D, it may be assumed for present purposes that substitution has a negligible effect on pairing in the hexaploid combinations, and that cytologically, though not genetically, the genome constitution of gametes from the A and D hexaploids is also equivalent to (AD)N.

PENTAPLOIDS

All of the nine hexaploids, 2(AD)N, have been crossed with *G. hirsutum*, 2(AD), to give pentaploids, (2(AD))N, with approximately 65 chromosomes. The pentaploids are more or less fertile on backcrossing to *G. hirsutum*, and their usefulness in introducing whole and part chromosomes from diploid species into *G. hirsutum* (BROWN 1949, 1951) will be dealt with in a later paper.

A brief discussion of their cytological behavior is necessary here, since some of the hybrids between hexaploids in certain respects may be thought of as "doubly pentaploid."

The maximum pairing between (AD) and N genomes in triploids is greatly reduced in hexaploids, probably because of preferential pairing and low chiasma frequency. Preferential pairing of the two (AD) genomes is still expected in the pentaploids, but since the N genome lacks homologues, increased (AD)–N pairing might also be expected. The expectation on this basis may be expressed as: $II_{3x} > III_{5x} > IV_{6x}$.

Table 3 summarizes the actual relations in series for which pentaploid analyses are available. From these data, it appears that the difference between III association in the pentaploids and IV frequency in the hexaploids is actu-

TABLE 3

Comparison of pairing in triploids, pentaploids and hexaploids, to test assumption that $II_{3x} > III_{5x} > IV_{6x}$.

Diploid genome	II/cell in (AD)N = 3x	III/cell in (2(AD))N = 5x	IV/cell in 2(AD)N = 6x
A_1	(13)	4.13–5.67*	0.63–5.19
A_2	(13)	3.53*	2.13–4.42
B_1	2.6–10.48**	1.79†	0.50–0.71
$D_{3\text{-}1}$	(13)	5.43†	4.43–4.90
$D_{3\text{-}2}$	(13)	3.80†	3.54
D_5	(13)	3.22†	4.34

*IYENGAR, 1943.
**See table 2. Maximum number of II expected is 13.
†Authors' data.

TABLE 4

Results of crosses between hexaploids. The notation "seeds" indicates good seeds have been obtained, but have not been planted. "R" indicates seeds or plants of the reciprocal cross have been obtained.

Ovule parent	Pollen parent								
	$2(AD)_1A_1$	$2(AD)_1A_2$	$2(AD)_1B_1$	$2(AD)_1C_1$	$2(AD)_1D_1$	$2(AD)_1D_{2-1}$	$2(AD)_1D_{2-2}$	$2(AD)_1D_3$	$2(AD)_1E_1$
$2(AD)_1A_1$	self	plants	plants	plants	R	plants	plants	plants	plants
$2(AD)_1A_2$	seeds	self	plants	plants	plants	R	plants	plants	plants
$2(AD)_1B_1$	seeds	seeds	self	plants	seeds	plants	seeds	plants	plants
$2(AD)_1C_1$	plants	R	seeds	self	R	R	R	plants	plants
$2(AD)_1D_1$	plants	plants	plants	plants	self	not tried	not tried	plants	plants
$2(AD)_1D_{2-1}$	plants	plants	plants	seeds	not tried		not tried	R	plants
$2(AD)_1D_{2-2}$	R	R	R	plants	not tried	not tried		R	R
$2(AD)_1D_3$	seeds	seeds	seeds	plants	R	seeds	plants	self	plants
$2(AD)_1E_1$	seeds	seeds	seeds	plants	plants	plants	plants	seeds	self

ally of doubtful significance. Absence of a homologous set of chromosomes apparently does not increase the ability of N to pair with (AD). Competition seems to operate in the formation of the characteristically low number of chiasmata seen at metaphase, so that partially non-homologous chromosomes are unable to compete effectively for chiasmata with the (AD) genome when a second (AD) genome is present. These considerations are germane to the analysis of the hexaploid combinations, since it may therefore be assumed that in (AD)N/(AD)M hybrids, pairing between (AD) and N, and (AD) and M, is no greater than it is in the respective hexaploid parents.

HEXAPLOID COMBINATIONS

HARLAND (1940) obtained all combinations of $2(AD)_2D_1$, $2(AD)_2D_{2\text{-}1}$ and $2(AD)_2D_4$. BROWN (1951) reported crosses of $2(AD)_1D_{2\text{-}1}$ with A_1, A_2, B_1, C_1 and E_1 hexaploids. IYENGAR (1944) intercrossed several hexaploids and obtained the following types hybrid for the diploid genomes: $(AD)_1A_1/(AD)_1B_1$, $(AD)_2A_1/(AD)_2D_{2\text{-}1}$ and $(AD)_2D_{2\text{-}1}/(AD)_1A_1$. Of 23 such plants, 16 had 78 chromosomes, and the remaining seven ranged from $2n = 76$ to $2n = 85$. Thirty-seven seedlings of $2(AD)_1D_{2\text{-}1}$ and $2(AD)_2D_{2\text{-}1}$ ranged from 74 to 82 chromosomes, with 26 having $2n = 78$. Of sixteen seedlings of $2(AD)_2B_1$ and $2(AD)_1B_1$, 12 had 78 chromosomes, three had 80 and one had 81. He concluded that gametes with 39 chromosomes function most frequently. None of these authors gives data on meiotic pairing in the hexaploid hybrids.

Crossing behavior

Table 4 shows the crosses obtained between hexaploids involving nine diploid species. Results of crossing these diploid species directly range from easily obtained, well-filled bolls with good seeds from which vigorous and even fertile progeny are obtained, through various levels of partial incompatibility, to complete failure of crossing (crossed flowers dropping as quickly as unpollinated flowers). All crosses between the hexaploids, however, are easily effected. Mature bolls with viable seeds giving vigorous hybrids are readily obtained. There is some variation in number of seeds per boll in various combinations, and crosses involving the $D_{2\text{-}1}$, D_5 and C_1 hexaploids are more easily procured by using these as the male parent. But both of these limitations are probably a function of the fertility of the hexaploid rather than of specific incompatibility. If a crossed boll shows signs of fertilization at all (rapid enlargement during the first ten days), it generally produces good seeds giving high germination and vigorous seedlings. The definite types of arrested development characteristic of various interspecific crosses, such as late falling of bolls, empty seeds, poor germination, weak or abnormal seedlings, are not typical of any hexaploid × hexaploid cross.

The hybrids thus produced include several combinations of diploid genomes which have not been previously reported (A_1D_5, B_1C_1, A_1E_1, $D_{2\text{-}1}E_1$, D_5E_1, D_5C_1, C_1E_1) or which die, in direct crosses, at a stage too young for meiotic analysis (D_1E_1, B_1E_1). In addition, two other crosses (A_2D_5, STEPHENS

1944, and B_1D_5, STEPHENS unpublished) have been obtained with difficulty but have not been analyzed cytologically. The B_1C_1, B_1E_1, C_1E_1 and DE_1 crosses are of particular interest since they bring together not only species but also genome groups whose cytological affinities have not previously been studied in direct crosses.

Fertility

Fertility tests of the hexaploid hybrids have not yet been completed. However, 17 different combinations, analyzed cytologically, have subsequently been maintained in the greenhouse for a year or longer. Sixteen of these, each combining diploid species from two different genome groups, have shown no indication of self-fertility. This is scarcely surprising, in view of the diversity and irregularity of meiotic behavior. Some of the *hirsutum–arboreum–herbaceum* hybrids, combining A_1 and A_2, however, appear to be somewhat self-fertile. None of the hybrids combining two D genomes have been grown long enough for a test of self-fertility.[3]

Although some of the hexaploids themselves and most of their corresponding pentaploids are virtually self-sterile, all are more or less fertile when crossed with *G. hirsutum*. The maximum fertility of the hexaploid combinations may therefore be more accurately assessed by crossing to *G. hirsutum*. Results of testing fourteen different combinations against *G. hirsutum* are summarized in table 5.

As will appear from table 5, the hexaploid combinations so far tested are very infertile with *G. hirsutum*. However, with persistent attempts a few seeds have been obtained from several combinations. Since the few bolls seem to be rather uniformly distributed with regard to the degree of relationship between the two diploid genomes involved, it is probable that a few offspring could at length be obtained from all the combinations. Not all the seeds produced have been planted. Plants grown so far were from excised embryos started on nutrient agar and later removed to pots in the greenhouse.

Five plants have been obtained from crosses using the hexaploid combinations as male parents. One, from *G. hirsutum* × $(AD)_1A_2/(AD)_1D_5$, resembled *G. hirsutum*, was fully fertile, and had 26 II at metaphase I in the pollen mother cells. The other four, from one boll, were from *G. hirsutum* × $(AD)_1B_1/(AD)_1C_1$. These also had 26 II at metaphase I and were fertile. It is probable that all five of these 52-chromosome plants resulted from contaminations, since unprotected flowers of the hexaploid hybrids were used for pollen. Since there is a possibility (see MENZEL and BROWN, 1952), however, that they represent rare types of gametes from the hexaploid hybrids, controlled crosses are being made using protected blossoms for pollen.

A number of embryos from the crosses of hexaploid hybrid female × *G. hirsutum* (table 5) germinated but gave weak and abnormal seedlings which died

[3] Since the manuscript was sent to press both the $(AD)_1D_1/(AD)_1D_5$ and $(AD)_1D_5/(AD)_1D_{5\text{-}5}$ hexaploid combinations have set probable selfed bolls in the greenhouse. Also, ten of eighteen plants in a field progeny of $(AD)_1A_2/(AD)_1E_1$ proved unexpectedly fertile following open pollination.

TABLE 5

Crosses to test fertility of hexaploid × hexaploid hybrids with G. birsutum.

	No. crosses	No. bolls	No. seeds*	No. plants	Remarks
$(AD)_1A_1/(AD)_1B_1 \times$ *birsutum*	30	4	25+	1	Lived two months
Reciprocal	27	0			
$(AD)_1A_2/(AD)_1B_1 \times$ *birsutum*	1	0			
Reciprocal	5	1	4 empty		
$(AD)_1A_2/(AD)_1C_1 \times$ *birsutum*	51	0			
$(AD)_1A_1/(AD)_1D_2 \times$ *birsutum*	81	0			
Reciprocal	6	0			
$(AD)_1A_2/(AD)_1D_2 \times$ *birsutum*	65	4	9	3	1 lived two months 1 lived six months 1 died early
Reciprocal	13+	3	3+	1	Had 26 II, fully fertile
$(AD)_1A_2/(AD)_1E_1 \times$ *birsutum*	28	0			
Reciprocal	8	0			
$(AD)_1B_1/(AD)_1C_1 \times$ *birsutum*	202	2	4	3	2 died early, one lived, was approximately 5 X.
Reciprocal	4	1	4	4	All had 26 II, fully fertile
$(AD)_1B_1/(AD)_1D_2 \times$ *birsutum*	16	0			
$(AD)_1B_1/(AD)_1E_1 \times$ *birsutum*	20	1	4		One additional open-pollinated boll gave a plant approximately 5 X.
Reciprocal	27	0			
$(AD)_1C_1/(AD)_1D_2 \times$ *birsutum*	18	0			
Reciprocal	1	1	1		
$(AD)_1D_2/(AD)_1C_1 \times$ *birsutum*	15	0			
$(AD)_1C_1/(AD)_1E_1 \times$ *birsutum*	65	1	2	2	One died early, one lived, approximately 5 X.
Reciprocal	10	0			
$(AD)_1D_1/(AD)_1B_1 \times$ *birsutum*	103	0			
Reciprocal	34	1	1		
birsutum $\times (AD)_1D_1/(AD)_1C_1$	16	0			
$(AD)_1D_2/(AD)_1E_1 \times$ *birsutum*	32	0			
Reciprocal	7	0			

*Not all the seeds have been planted.

before or shortly after transplanting. Only three seedlings have lived long enough for analysis: one from $(AD)_1B_1/(AD)_1C_1$, one from $(AD)_1C_1/(AD)_1E_1$, and one from an open-pollinated boll on a field-grown plant of $(AD)_1B_1/(AD)_1E_1$. All three of these plants had approximately the pentaploid chromosome number. The morphology of each indicated that some characters from the diploid genomes of the hybrid parent, such as petal color or leaf shape and texture, had been transmitted. These plants were self-sterile, and their fertility with *G. hirsutum* has not been adequately tested. However, examination of meiosis indicated that two more or less complete and regularly pairing (AD) genomes, plus 13 ± univalents from the diploid genomes, were present. Some trivalents, but no higher associations, were found. It is probable that these plants would, if tested extensively, show some fertility with *G. hirsutum*, since their meiotic behavior is similar to that of the pentaploids.

TABLE 6

Average pairing in hexaploid × hexaploid hybrids.

Combination	II	III	IV	V	VI	VII	VIII	IX	X	XII	I	Av. paired	Av. MN pairing	Max. MN pairing	No. cells
$(AD)_1A_1/(AD)_1A_2$	24.56	1.46	5.13	0.13	0.05	0.03	0.03				2.16	75.46	10.81	13	37
$(AD)_1A_1/(AD)_1B_1$	22.68	4.20	2.34	0.55	0.09	0.07	0.05	0.05	0.02		5.89	72.09	7.43	10	44
$(AD)_1A_2/(AD)_1B_1$	20.07	6.34	1.72	0.34	0.07	0.03					9.41	68.45	4.79	9	29
"	22.61	3.96	2.19	0.54	0.23	0.08				0.04	6.80	70.96	7.07	10	26
$(AD)_1A_2/(AD)_1C_1$	23.19	5.27	1.00		0.07						11.11	66.65	4.30	8	26
$(AD)_1A_1/(AD)_1D_5$	17.33	10.16	0.12	0.16							11.16	66.50	2.12	5	24
$(AD)_1A_2/(AD)_1D_5$	14.73	11.57	0.61	0.19	0.35						8.04	69.84	2.61	5	26
$(AD)_1D_1/(AD)_1A_2$	15.94	8.90	1.28	0.44	0.09	0.06	0.03	0.03			10.50	67.40	2.81	7	32
$(AD)_1A_1/(AD)_1E_1$	20.00	5.50	1.27	0.19	0.11	0.04					13.38	63.50	2.77	6	26
$(AD)_1A_2/(AD)_1E_1$	20.08	5.97	0.67	0.20	0.03	0.03					15.85	62.20	2.00	6	34
$(AD)_1B_1/(AD)_1C_1$	25.92	1.50	0.19								19.27	58.27	2.19	9	26
$(AD)_1B_1/(AD)_1D_5$	19.27	7.70	0.75	0.05	0.07						12.22	65.35	2.70	5	40
$(AD)_1D_1/(AD)_1B_1$	20.34	7.05	0.30	0.05							14.28	63.33	2.07	4	39
$(AD)_1B_1/(AD)_1E_1$	25.55	1.90	0.05								20.55	57.07	1.55	6	54
$(AD)_1C_1/(AD)_1D_5$	19.23	8.38	0.15	0.04							13.46	64.42	2.00	5	26
$(AD)_1D_1/(AD)_1C_1$	22.22	5.24	0.55	0.04							14.43	62.53	1.73	5	53
$(AD)_1C_1/(AD)_1E_1$	26.76	0.72	0.20								21.16	56.48	1.88	5	25
$(AD)_1D_5/(AD)_1E_1$	21.32	6.21	0.10								15.89	61.71	1.68	4	28

Cytological observations

Chromosome pairing was studied in temporary acetocarmine pollen mother cell smears. The high number of small chromosomes and irregularity of pairing in these hybrids made it difficult to determine the exact number of chromosomes in many cells. In especially favorable material, it was ascertained that two plants had $2n = 77$. Most of the rest had 78, and all fell within the range $2n = 78 \pm 2$. In computing average pairing, cells were included in which 76–80 chromosomes were counted. Since the greatest inaccuracy lay in counting numerous univalents scattered around the periphery of the plate, usually off the spindle, it is felt that this source of error does not unduly prejudice comparisons of pairing behavior.

Average pairing in 17 different combinations of hexaploids is summarized in table 6. Table 7 shows the range in number of univalents and different types of association for each hybrid.

TABLE 7

Range in number of univalents and different kinds of associations in hexaploid × hexaploid hybrids.*

Combinations	II	III	IV	V	VI	I
$(AD)_1A_1/(AD)_1A_2$	16–31	0–5	1–9	0–1	0–1	0–5
$(AD)_1A_1/(AD)_1B_1$	15–29	1–9	0–6	0–3	0–2	0–13
$(AD)_1A_2/(AD)_1B_1$	17–28	1–7	0–6	0–3	0–1	3–10
"	13–28	2–11	0–4	0–2	0–1	3–17
$(AD)_1A_2/(AD)_1C_1$	17–29	2–10	0–2			6–20
$(AD)_1A_1/(AD)_1D_5$	9–25	3–17	0–1	0–1		3–21
$(AD)_1A_2/(AD)_1D_5$	9–24	4–17	0–2	0–1	0–2	5–13
$(AD)_1D_1/(AD)_1A_2$	9–23	5–14	0–4	0–2	0–1	2–17
$(AD)_1A_1/(AD)_1E_1$	14–28	0–10	0–3	0–1	0–1	7–20
$(AD)_1A_2/(AD)_1E_1$	14–26	2–11	0–2	0–1	0–1	10–20
$(AD)_1B_1/(AD)_1C_1$	21–32	0–7	0–2			3–27
$(AD)_1B_1/(AD)_1D_5$	15–27	3–13	0–3	0–1	0–1	6–17
$(AD)_1D_1/(AD)_1B_1$	15–25	2–12	0–2	0–1		8–18
$(AD)_1B_1/(AD)_1E_1$	20–32	0–5	0–1			10–29
$(AD)_1C_1/(AD)_1D_5$	9–26	3–10	0–1	0–1		6–17
$(AD)_1D_1/(AD)_1C_1$	17–26	2–11	0–2	0–1		9–21
$(AD)_1C_1/(AD)_1E_1$	22–31	0–3	0–1			14–30
$(AD)_1D_5/(AD)_1E_1$	16–26	4–10	0–2			12–24

*Associations of more than six chromosomes are omitted in this table, since they are rare and never more than one per cell.

The most striking feature of meiosis in these hybrids is the great variation in pairing from cell to cell in the same plant. For instance, 54 cells of $(AD)_1B_1/(AD)_1E_1$ showed 40 different configurations, only 9 of which were found more than once. Yet pairing in this hybrid was the simplest observed, three cells having IV's, and all the rest only II's, III's and I's. Similarly, $(AD)_1D_1/(AD)_1C_1$ showed 38 different configurations in 53 cells, with only eleven repeated twice or more. Inspection of table 7 shows that the variation in number of unpaired chromosomes was also quite wide, except for $(AD)_1A_1/(AD)_1A_2$, which had a maximum of 5 I's in two of the 37 cells analyzed, while only one cell showed all chromosomes paired.

Anaphase separation was clean for the most part, although occasional bridges or bridge-fragment figures were observed at anaphase or telophase. Unequal one-chiasma bivalents, and less commonly, trivalents or higher multivalents composed of chromosomes differing markedly in size, were observed in 12 of the 17 combinations (figs. 1 and 3).

Metaphase ring associations of three chromosomes were observed in two combinations, one in $(AD)_1D_1/(AD)_1B_1$ (fig. 2), and two in $(AD)_1D_1/(AD)_1C_1$. One of the latter had a fragment lying near it. These rings of three are interpreted as evidence of segmental interchange in the differentiation of the various genomes, the pairing being considered to be of the $\begin{smallmatrix} & 22 & \\ / & & \backslash \\ 11 & - & 33 \end{smallmatrix}$ type.

For the most part, bivalents and other associations were normal in appearance. Precocious or attenuated bivalents (usually with only one chiasma), and less often, attenuated trivalents and quadrivalents, were seen in cells from several hybrids (figs. 1 and 2). These were often, though not always, associations of large and small chromosomes; however, not all asymmetrical associations were attenuated. These figures occurred in cells in which most of the associations appeared normal.

There is a characteristic size differentiation between the meiotic metaphase chromosomes of different genomes in Gossypium. While the absolute size difference is not constant from plant to plant, or from cell to cell on the same slide, the relative sizes within a cell may often be used, within limits, as a clue to the type of pairing in plants of known genome constitution. Thus, all of the D genomes have relatively small chromosomes, those of D_5 being the smallest in this group, while the A and B genomes are large and those of C and E relatively very large.

Table 8 shows the type of pairing in six especially clear metaphase plates of an $(AD)_1D_1/(AD)_1C_1$ plant with $2n = 77$. A plant of this constitution is expected to have 39 small (D_h and D_1), and 39 large to very large (A_h and C_1) chromosomes. One to two 2-chiasma bivalents per cell were intermediate in size, and these were considered arbitrarily to be D pairs. Most of the I's were very large; three cells had two somewhat smaller univalents (which were nevertheless larger than the smaller bivalents), and these were classified as "large" chromosomes. All other configurations could be classified clearly as large, small or a mixture of large and small chromosomes. Hence there is a possible maximum subjective error in size designation of six chromosomes. But since these do not involve multivalents or asymmetrical bivalents, the deduced pairing relations are probably reliable.

It will appear from table 8 that in this plant, one of the expected 39 small D chromosomes is missing, giving $2n = 77$. An average of 2.14 associations per cell between large and small chromosomes is found, and these are probably mostly between D and C_1. Some pairing of C_1 with A_h also occurs, probably averaging between one and two chiasmata per cell in these six cells. These cells showed average pairing of 22.83 II, 5.00 III, 0.50 IV and 13.50 I. The

FIGURE 1.—Metaphase I in $(AD)_1B_1/(AD)_1E_1$ with 25 II, 3 III, 19 I. Note attenuated bivalents, some of which are unequal.

FIGURE 2.—A portion of a metaphase I plate from $(AD)_1D_1/(AD)_1B_1$ showing attenuated configurations and a ring III.

FIGURE 3.—A metaphase I plate from $(AD)_1A_1/(AD)_1E_1$ with 18 II, 9 III, 14 I, showing size differentiation.

FIGURE 4.—Idiogram constructed from the plate shown in figure 3. Interpretation in the text.

TABLE 8

Pairing in six pollen mother cells of $(AD)_1D_1/(AD)_1C_1$ with good size differentiation between large A_h and C_1, and small D_h and D_1 chromosomes.

Configuration				Excess II-equiv.*	Pairing of large (L) and small (S) chromosomes											Total	
					II			III				IV		I			
II	III	IV	I		SS	SL	LL	SSS	SSL	LLS	LLL	SSSL	LLLL	S	L	S	L
20	7	1	12	3	7	1	12	6	1				1	3	9	38	39
23	5		16	2	8	2	13	5						5	11	38	39
23	6		13	3	10	1	12	4		1	1			4	9	38	39
23	4		19	1	9		14	4						8	11	38	39
24	4	1	13	4	11	1	12	2	2				1	5	8	38	39
24	4	1	13	3	9	2	13	3	1			1		4	9	38	39
Total 137	30	3	81	16	54	7	75	24	4	1	1	1	2	29	57		
Av. 22.83	5.00	0.50	13.50	2.66	9.00	1.16	12.50	4.00	0.66	0.16	0.16	0.16	0.33	4.88	9.50		

Av. SL pairing:
SL II 1.16
SSL III 0.66
LLS III 0.16
SSSL IV 0.16
2.14

The difference between 2.66 average excess II-equivalents* and 2.14 SL associations is probably due to pairing of A_h and C (LLLL IV and LLL III).

*See text for definition.

overall average of 53 cells was 22.22 II, 5.24 III, 0.55 IV, 0.04 V and 14.43 I. Hence pairing in the smaller sample is probably representative. The calculated average of 2.14 DC associations per cell is only slightly higher than the estimated average of 1.73 D_1C_1 chiasmata per cell arrived at in a different way (see later).

Figure 3 pictures a metaphase plate with 18 II, 9 III, and 14 I from an $(AD)_1A_1/(AD)_1E_1$ hybrid which had $2n = 77$. Figure 4 is an idiogram made from this plate. Reading from bottom to top on the idiogram, pairing in this cell was as follows:

	Chromosomes of genomes		
	D	A	E
12 small II's	24		
6 large II's		12	
7 large III's		21	
1 III = small II + large attached chromosome	2		1
1 III = large II + very large attached chromosome		2	1
14 large I's		3	11
TOTAL	26	38	13

On this interpretation, one A chromosome is missing. This is also the most likely probability, since the A_1 hexaploid parent is more likely to show irregular segregation than the E_1 hexaploid (see table 1). According to the above analysis there is one A_1E_1 chiasma present, which is demanded by the presence of 27 associations in the plate. E_1 also forms one chiasma with D_h. The two intergenomic chiasmata both occur in trivalents in this cell.

ESTIMATED PAIRING BETWEEN DIPLOID GENOMES

The principal interest in meiotic pairing in the hexaploid combinations lies in the opportunity they afford for estimating the homology between the diploid genomes. If the two diploid genomes are entirely non-homologous, the pairing will resemble that of a "double pentaploid": the diploid genomes will each form a number of III's with (AD), of similar magnitude to the number of IV's formed in the corresponding hexaploid, and the rest of the chromosomes will appear as I's. On this basis, the number of I's expected in the hexaploid hybrid can be computed by the formula: $26 - (IV_{6x_N} + IV_{6x_M}) = I_{6x_{MN}}$. The disparity between expected and actual frequency of I's so computed provides a rough estimate of pairing between the two diploid genomes. By this method, pairs of genomes may be arranged in order of their affinity, those showing the greatest divergence from expectation for a "double pentaploid" being most closely related.

The *actual* amount of pairing between two diploid genomes cannot, however, be accurately estimated by the "double pentaploid" method, because most hexaploids and pentaploids (and, by inference, hexaploid combinations) show multivalents with (AD) other than IV's and III's respectively, and the exact composition of these is more difficult to deduce.

A better estimate of the average NM pairing can be obtained in the following manner: If only (AD) II's, (AD)N III's and (AD)M III's are formed, the maximum number of associations possible is 26. The excess above 26 is taken as the number of NM associations. The error due to the occurrence of associations of more than three chromosomes is corrected for as follows:

II's and III's are counted as one association.

IV's, V's and VI's are counted as two associations (equivalent to 2 II's, 1 II + 1 III, 2 III's respectively).

VII's, VIII's and IX's are counted as three associations, etc. The corrected estimate of II and III associations above 26 is termed " excess II-equivalents " (table 8 and supplementary tables).

Some error may still accrue to formation of rarer types of associations: *e.g.*, if a IV or two II's of composition NA_hA_hM (or $ND_h + D_hM$) are formed, or if two homologous (AD) chromosomes each pair as II's with two different N or M chromosomes, $D_hN_1 + D_hN_2$, 27 associations could be formed without NM pairing. This error could be corrected by considering the frequency of 14 + associations per cell and of III formation in the (AD)N triploid hybrids, but since these are rare in triploids, it is assumed that the error is negligible.

Table 9 compares the estimates of average and maximum NM pairing obtained by the above method for the hexaploid combinations, with pairing in the corresponding F_1 diploid hybrids. It may be seen from table 9 that where pairing in two species combined in a hexaploid hybrid has also been studied in the corresponding diploid hybrid, the average pairing is considerably lower in the hexaploid than in the diploid. In general, however, the estimated *maximum* NM pairing in the hexaploid is close to the highest *average* bivalent formation reported for the diploid hybrid. The only striking exception is $C_1 \times D_1$. WEBBER reports an average of only 0.74 II for C_1D_1, while the estimated average in $(AD)_1C_1/(AD)_1D_1$ is 1.73 and the maximum 5. SKOVSTED (1937) gives averages of 7.65 and 4.75 II for the analogous hybrids $C_1D_{2\text{-}1}$ and C_1D_3. Since WEBBER's figures are also in disagreement with those of other investigators for A_2D_1 and A_2E_1, as well as for several hybrids not here considered, it seems likely that C_1D_1 usually exhibits more pairing than he found.

It may thus be predicted empirically that pairing in species combinations hitherto not obtained directly, would, if they could be obtained, average near the maximum found in the hexaploid hybrid. Thus:

1. A_1D_5 and A_2D_5 should average about 5 II. This agrees fairly well with pairing in analogous A_2D_1 (SKOVSTED, IYENGAR, unpublished data of the authors).

2. A_1E_1 should average about 6 II, which is in good agreement with the findings for A_2E_1.

3. B_1D_5 should average about 5 II. This is close to SKOVSTED's figure for B_1D_1 and BROWN's (1951) for B_1D_3, and somewhat higher than those for B_1D_3 reported by SKOVSTED (1937), BEASLEY (1942) and WEBBER (1939), and for B_1D_4 (SKOVSTED 1937).

4. C_1D_5 should form about 5 II, similar to $C_1D_{2\text{-}1}$ and C_1D_3 (SKOVSTED 1937). The estimate for the $(AD)_1C_1/(AD)_1D_5$ hexaploid is close to that for the $(AD)_1C_1/(AD)_1D_1$ hybrid, both of which are much higher than the average reported by WEBBER for C_1D_1, as mentioned above.

Similar predictions may therefore be made for the combinations of genome groups for which comparable diploid hybrids have not been studied because of failure to cross or early death of the hybrids.

TABLE 9

Comparison of pairing between diploid genomes combined in hexaploid hybrids and in diploid hybrids.

Combination of diploid genomes	Estimated pairing in hexaploid hybrid		Pairing in corresponding diploid F_1 (Average II)	Author
	Average	Maximum		
A_1A_2	10.81	13	usually all paired	(See text)
A_1B_1	7.43	10	11.75	Skovsted 1937
A_2B_1	4.79	9	10.7 (+multivalents)	Iyengar 1942
"	7.07	10	11.82	Webber 1939
			10.45–11.55 (+multivalents)	Skovsted 1937
A_2C_1	4.30	8	0–8	Webber 1936
			9.5–10.2 (+multivalents)	Skovsted 1937
A_2D_1	2.81	7	6.35–9.56	Skovsted 1937
			0.2	Webber 1938
			1.02	Webber 1939
			2–6	Iyengar 1943
A_1D_5	2.12	5	no report	
A_2D_5	2.61	5	no report	
A_1E_1	2.77	6	no report	
A_2E_1	2.00	6	7.13	Abraham 1940
			4.35–7.05	Skovsted 1937
			3.8	Beasley 1942
			0.95	Webber 1939
B_1C_1	2.19	9	no report	
B_1D_1	2.07	4	5.7	Skovsted 1937
B_1D_5	2.70	5	no report	
B_1E_1	1.55	6	one PMC had 5 II 16 I	J. O. Beasley unpublished
C_1D_1	1.73	5	0.74	Webber 1939
C_1D_5	2.00	5	no report	
C_1E_1	1.88	5	no report	
D_5E_1	1.68	4	no report	

5. B_1C_1 should form about 9 II; there is, therefore, nearly as much homology between B_1 and C_1 as between A and B, or A and C.

6. B_1E_1[4] should show about 6 II; C_1E_1, about 5 II; D_5E_1, about 4 II. E_1 is therefore about equally distinct from A, B, C and D. (One cell analyzed by BEASLEY in an F_1 hybrid of B_1E_1 had 5 II and 16 I (see STEPHENS 1950).)

[4] Since this paper was prepared, DOUWES (1951) has published an analysis of pairing in B_1E_1. An average of 2.7 II was found in twenty-three cells, which is lower than the present estimate. However, one cell had 6 II, and seven had 4–5 II.

CONCLUSIONS

By the methods described, it has been possible to study directly the cytological affinity of each genome group in Gossypium with every other genome group. The relationships thus established are, in general, in excellent agreement with the inferences of previous investigators from less direct evidence. Since these have been assembled by HUTCHINSON, SILOW and STEPHENS (1947), they need not be discussed further here.

Whatever their nature, crossing barriers between certain diploid species are overcome in the hexaploids, and to a lesser extent in other combinations yet to be described. Conclusions regarding the crossability of the diploid species and the relation of crossability to ploidy and to genome balance (STEPHENS 1942, 1945) will be discussed later, when data on all species and hybrid combinations have been presented.

The chief interest in the present study lies in the demonstration that at least some chiasmata may form between the chromosomes of any two genome groups in the genus, even between those groups that are most distantly related. Hence it may be assumed that exchange of chromatin is *cytologically* possible among all genome groups, even though the exchange in many cases may be limited to parts of only a few chromosomes of the complement. Interspecific transfer of chromatin is, of course, severely limited by the fertility of hybrids in which such exchanges occur at meiosis, as well as by numerous other practical considerations. It may be concluded, however, that at least some of the genic material of species in every diploid genome group of the genus is theoretically available for transfer, directly or indirectly, into the cultivated cottons. The hexaploid trispecies hybrids have been used to establish this point, since this type of cross yields the greatest number of different combinations of diploid genomes. However, many of these genomes may also be combined in hybrids at a lower level of ploidy. It is probable that some of the other series of polygenomic hybrids will prove more promising for the practical transfer of chromatin between distantly related genomes.

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GENETIC AND NON-GENETIC FACTORS IN PIGMENTATION OF NEUROSPORA CRASSA

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THE biochemical genetics of carotenoid pigments has been studied in the tomato (LEROSEN *et al.* 1941; MACKINNEY and JENKINS 1949; PORTER and LINCOLN 1950), corn (MANGELSDORF and FRAPS 1931) and red yeast (BONNER *et al.* 1946). HAXO (1949) worked out the carotenoid components of the wild type Neurospora. The present paper deals with genetic and biochemical studies of a modifier system for pigmentation in Neurospora and the effect of light on this system.

MATERIALS AND METHODS

In the genetic analysis, spores were isolated on complete medium including nutritionally normal strains, since unmistakable judgment of all the color types can be made only on complete medium. For biochemical studies of pigments four nutritionally wild-type color types of the same mating type *a* from a cross of a valine-isoleucineless mutant 16117*A* to albino 15300*a* were used. The medium for mass culture was HAXO's enriched medium (HAXO 1949). 3L-Fernbach flasks containing 300 ml of agar medium were used for mass culture. Mold from 5 to 15 such flasks was used for each experiment depending upon the pigment content of the strain. The cultures were incubated for four days at 25°C in a dark room, followed by ten days at room temperature (25 ± 2°C) illuminated with a 14 watt daylight fluorescent lamp at a distance of 50–60 cm.

The procedure of extraction and chromatography adopted by HAXO (1949) was followed except for minor modifications. Extraction was done with the aid of a Waring blender. The residue was saved for dry weight determination. The same amount of acetone was used in the extraction for all the color types irrespective of their color intensity taking the most intensely colored type as a standard.

Columns of size 35 × 250 mm and 20 × 200 mm were used for chromatography. Hyflo super cel instead of celite was used as the filter aid. Identification and quantitative estimation of the carotenoids were made exclusively with a Beckman spectrophotometer.

GENETICS

When spores from crosses of the valine-isoleucineless 16117*A* or the wild-type *crassa* 1*A* to the albino strain 15300*a* were isolated on complete medium,

[1] This work was done during the tenure of a Public Health Fellowship sponsored by the National Cancer Institute, Federal Security Agency.

asci of two and four color types could be distinguished in the fully grown cultures. In the latter the color types were salmon (the wild-type color), pink, peach and white (the albino color). In the former they may be either salmon and white or pink and peach. A two factor hypothesis was proposed. Accordingly the genotype of the parents may be represented as *C* (or $+^{al_2}$, the major color gene) *I* (the intensifier) and *c* (or al_2) *i*. The four color type ascus therefore would be the tetratype and the two-color type ascus, the parental and non-parental ditypes (PERKINS 1949).

This hypothesis was tested by making further crosses. Thus, crosses of *cI* × *CI*, *ci* × *Ci* and *ci* × *cI* gave exclusively ditype in eleven asci, while the cross of *Ci* × *cI* gave one tetratype in two asci.

An analysis of 51 asci, 6 from a cross of *crassa* 1*A*, 35 from valine-isoleucineless 16117*A* to the albino strain 15300*a*, and 10 from Chilton *a* to 15300*A*, gave the centromere distance for the *C* gene as 25 map units, as compared with the published value 24.6 (HOULAHAN *et al.* 1949), and that of the *I* gene 20 map units.

Among 41 asci the numbers of tetratypes, parental and non-parental ditypes were 23, 11 and 7, respectively. This indicates that *C* and *I* are not linked. If two genes are linked then the number of asci of parental ditype should be more than that of non-parental ditype, because both noncrossover and two strand doubles would give rise to the parental ditype while only four strand doubles would give rise to the non-parental ditype. Conversely, if two genes are not linked then the parental and non-parental ditypes should always be equal, and the number depends upon the centromere distance (PERKINS 1949 table 3).

ANALYSIS OF PIGMENTS

A preliminary experiment was carried out with the wild type *crassa* 1*A*. The preliminary chromatogram and the chromatogram of the major sections were in essential agreement with the results of HAXO (1949). One extra component was identified tentatively by its absorption maxima and minima (max. 377, 397, 422, 452; min. 385, 410, 445) as pigment B described in the red yeast (BONNER *et al.* 1946, max. 377, 399, 424; min. 386, 411). On the preliminary chromatogram it was barely visible on the column lying between δ-carotene (sec. D, table 1) and β-carotene (sec. F). No quantitative estimation of the pigments in *crassa* 1*A* was attempted.

The colors of the extracts of the four types, *CI*, *Ci*, *cI* and *ci*, were golden yellow, reddish orange, red, and light yellow. The results of the chromatograms of the epiphase of the four types before developing further with 3 percent acetone in petroleum ether are shown in table 1, the *CI* type being identical with that of *crassa* 1*A*.

The major sections of the *CI* type after rechromatography again gave chromatograms essentially identical with those of *crassa* 1*A*. The quantity of pigments eluted from the chromatograms of all the color types is shown in table 2. The quantities of isomers which could be the results of isomerization in the

TABLE 1

Major sections of chromatograms of the epibase of the four color types. Figures in parentheses represent the breadth of the section, in mm.

Major section (major pigment)	CI	Ci	cI	ci
A (spirilloxanthin)	rose red (3)	same (3)	faint red (3)	faintly colored streak[1]
B (lycopene)	reddish orange (8)	same (3)		
C (neurosporene)	lemon yellow (9)	same (6)	faint yellow (5)	....
D (γ-carotene)	orange (5)	same (4)	same (1)	....
(δ-carotene)	faint red (14)	....	....	....
	colorless (15)	same (20)	same (20)	....
E (pigment B)	very faint yellow (7)	same (7)	....	....
	colorless (20)	same (35)		
F (β-carotene)	pale orange (15)	same (10)	very faint (10)	....
G (phytofluene)	fluorescent (20)	same (15)	....	....

[1] No movement of the streak occurred on prolonged development with 3 percent acetone in petroleum ether.

TABLE 2

Carotenoid content of four color types of N. crassa grown in the light. Figures represent mg carotenoid per 100 gm dry wt. of mold with the exception of δ-carotene, pigment B and the acidic hypophasic pigment, which are in relative concentrations. Figures in parentheses represent dry wt. of mold used in each experiment.

Carotenoid	CI (22.5 gm)	Ci (21 gm)	cI (42 gm)	ci (45 gm)[4]
Spirilloxanthin	2.45	0.19	....	....
(Neo-A)	(0.25)	(0.08)		
Lycopene	3.72	0.14	....	....
(Neo-A)	(0.42)	(0.05)		
Neurosporene	5.0	0.23	....	....
γ-Carotene	8.8	0.50	0.024	....
β-Carotene	0.99	0.04	0.011	....
Sum	20.96	1.10	0.035	....
Phytofluene	2.5	0.41	....	....
	2.6[2]	0.27[3]		
δ-Carotene[1]	1.43	0.15	....	....
Pigment B[1]	12.2	1.26	....	....
	17.6[2]	0.55[3]		
Hypophase[1]	10.91	5.66	0.84	....
Relative amt. of epiphasic pigments	1	1/10–1/28	1/90–1/600	....

[1] Relative concentration expressed in volume of the pigment × dilution × E, at the main peak/dry wt. of the mold.
[2] Dry wt. of the mold 9.2 gm in duplicate experiment.
[3] Dry wt. of the mold 7 gm in the duplicate experiment.
[4] Dry wt. of the mold 38 gm in the duplicate experiment.

experimental processes have been added to the main pigments and are indicated in parentheses. Only the main component of the hypophase is included in the table. The blanks indicate the absence of pigment. It is evident from the data presented in table 2 and our knowledge about the position of genetic blocks, which will be discussed later, that both the main color gene and the modifier have similar effects, though different in magnitude, and that their joint effect is non-additive.

EFFECT OF LIGHT ON THE CAROTENOID CONTENT

The wide occurrence of the colorless carotenoid phytofluene in plants is of interest because of its possible role as an intermediate in the biosynthesis of carotenoid pigments (ZECHMEISTER and SANDOVAL 1945; BONNER *et al.* 1946). HAXO (1949) reported a two-fold decrease of total carotenoid pigments and a slight increase in phytofluene when the cultures were kept in darkness. It was observed that cultures of the *CI* type wrapped in tin foil appeared almost colorless. Experiments were performed, therefore, to test the effect of complete darkness on the carotenoid content of the *CI* strain. The culture flasks were painted with aluminum paint, wrapped in black cloth and kept in a closed cabinet. The results are shown in table 3.

TABLE 3

Carotenoid content of cultures grown in light and in darkness. Figures represent mg carotenoid per 100 gm dry weight of mold. Figures in parentheses represent dry weight of the mold used in each experiment.

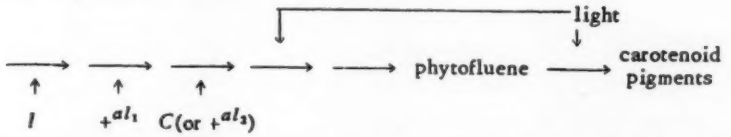
Carotenoid	*crassa* 1A (*Haxo* 1949) (Average of two lots)			CI (single lots except those marked with superscript one.)		
	In light	In dark	Ratio	In light (22.5 gm)	In dark (39.5 gm)	Ratio
Spirilloxanthin	6.60	1.95		2.45	0.13	
Lycopene	9.40	4.35		3.72	0.06	
Neurosporene	5.55	5.95		5.00	0.47 0.30[2]	
γ-Carotene	7.60	4.30		8.80	0.15	
β-Carotene	0.33	0.15		0.99	0.02	
Sum of pigments	29.48	16.70	0.57	20.96	0.83	0.04
Phytofluene	2.95	3.70	1.25	2.50 2.60[1]	1.98 1.30[2]	0.79
Sum of total carotenoids	32.43	20.40		23.46	2.81	
Ratio $\frac{\text{pigments}}{\text{phytofluene}}$	10.0	4.5		8.4	0.4	

[1]Dry weight of the mold 9.2 gm in duplicate experiment.
[2]Dry weight of the mold 8.5 gm in the duplicate experiment.

DISCUSSION

It has been reported that the biosynthesis of phytofluene runs parallel with the gradual formation of carotenoid pigments during the ripening of Pyracantha berries (ZECHMEISTER and SANDOVAL 1945). Attempts to demonstrate the accumulation of phytofluene in slightly colored or colorless mutants of red yeast met with failure (BONNER *et al.* 1946). This may be due to early genetic blocks before the formation of phytofluene, as suggested, and seems to be true also for the present case.

An analysis of the data listed in table 3 strongly supports the notion that the phytofluene could be the precursor of the carotenoid pigments. The agreement between our values and those of BONNER *et al.* on cultures grown in the light is reasonably close. The low values from our dark experiments could be due to the complete absence of light in our experiments. There is an increase of 25 percent in the amount of phytofluene in dark over that in light when the interference in the pigment formation is moderate, as in HAXO's experiments. In our experiments the accumulation of phytofluene is not apparent; in fact, there is a 22 percent decrease in absolute amount. However, when we take into consideration the tremendous decrease in the total amount of carotenoids and the change in the ratio of pigments to phytofluene from 8.4 to 0.4, the relative amount of phytofluene increases by a factor of 21. In short, light affects not only the conversion of phytofluene to carotenoid pigments but also, to a certain extent, the formation of phytofluene or its precursor just as the genes *C* and *I* do. A single experiment also was carried out on the non-allelic albino strain al_1 (4637*A*). Neither pigment nor phytofluene could be found in the extract of 8 gm dry weight of the mold. The following scheme of the biosynthesis of carotenoid pigments involving the genetic and non-genetic factors is therefore proposed.



The sequence of the genes $+^{al_1}$, *C* ($+^{al_2}$) and *I* and the effect of light before the formation of phytofluene is purely arbitrary and a branching scheme is not excluded. The location of genetic blocks of gene *c* and *i* before phytofluene, but not after, is further supported by the fact that the decrease of amount of different pigments is of the same order (table 2). The present study supports the scheme of biosynthetic pathways of carotenoids proposed by PORTER and LINCOLN (1950). The different magnitude of effect on the hypophase is not explained.

SUMMARY

The genetic data show that the four-color asci found in certain crosses can be explained by a *C* (major color gene)–*I* (intensifier) system. Experimental

data show that the more interference with the formation of carotenoid pigments, the greater the amount of phytofluene formed, relative to the amount of pigment when the cultures were grown in darkness. Phytofluene, therefore, is very likely a precursor of carotenoid pigments. Because of the fact that in the four-color types phytofluene runs parallel with pigment content, the genetic block associated with the genes *c* (al_2), *i* and al_1 must occur before the formation of phytofluene.

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THE GENETIC CONSEQUENCES OF ANAPHASE BRIDGE FORMATION IN DROSOPHILA

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CROSSING over between ring and normal X chromosomes of *D. melanogaster* was first studied by L. V. MORGAN (1933); the later work of STURTEVANT and BEADLE (1936) provided an understanding of the behavior of anaphase bridges resulting from crossing over in inversion heterozygotes. The present work represents an attempt to interpret the data from ring/rod heterozygotes using the rules derived from inversion heterozygotes by the above workers, with emphasis on a discrepancy between the observed and expected frequencies of that class of gamete with no X chromosome, the nullo-X class. It will be shown that this discrepancy, a deficiency of the nullo-X class, occurs also in experiments involving certain types of X inversion heterozygotes, and the bearing of the phenomenon on the nature of chromatid separation will be discussed.

CONSEQUENCES OF CROSSING OVER IN THE RING/ROD HETEROZYGOTE

The genetic consequences of crossing over in ring/rod heterozygotes are shown in figure 1. In the single exchange tetrad (E_1), a first anaphase tie insures the passage of a noncrossover chromatid, either a ring or rod, to the egg nucleus. The two-strand double exchange class (E_2-2s) gives rise to rings and rods, of both crossover and noncrossover types; all four should be recovered equally frequently. One of the two types of three-strand double exchange (E_2-3sa) produces a crossover rod chromatid, and a noncrossover ring, with a first anaphase bridge insuring the passage of either one or the other to the egg nucleus. The other type of three-strand two exchange tetrad (E_2-3sb) forms a tricentric complex, with a free noncrossover rod. Finally, the four-strand double class (E_2-4s) gives rise to a double chromatid bridge at first anaphase which, like the double bridges resulting from crossing over within inversion heterozygotes, would be expected to prevent the passage of an X chromatid to the egg. It is from this class that the nullo-X eggs are assumed to be derived.

The ratio of crossover rod chromatids to nullo-X eggs originating in two exchange tetrads will provide the theoretical expectation with which the observed results may be compared. If all four types of two exchange tetrads

[1] This work was carried out at the CALIFORNIA INSTITUTE OF TECHNOLOGY, aided by a contract with the Atomic Energy Commission operating through the Office of Naval Research, Department of Navy.

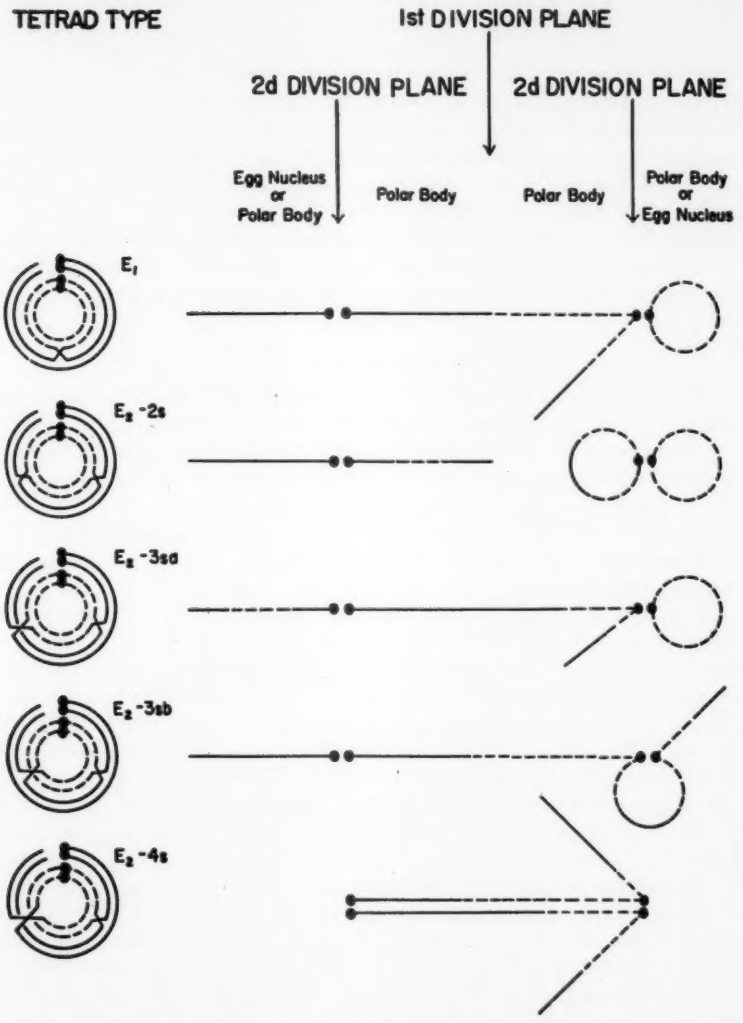

FIGURE 1.—The tetrad analysis of ring/rod heterozygotes showing the consequences of single and double exchanges. First anaphase bridges direct monocentric chromatids into the outermost products of the two meiotic divisions; either product may be the egg nucleus. Each of the four chromatids from the two-strand class has the same probability of being included in the functional egg nucleus.

occur with equal frequency, there will be four eggs from each type out of every 16 eggs derived from two exchange tetrads. One of the four eggs from the two-strand double class and two from the type E_2-3sa should include a crossover rod chromatid. All four eggs from the four-strand double class should be nullo-X; of these, half will be fertilized by X-bearing sperm. The ratio of recovered crossover rod chromosomes to patroclinous males should, therefore, be 3:2. Nothing definite can be said at this point about the expectation from the tetrad type E_2-3sb, since the tricentric might act as a first anaphase bridge, producing a nullo-X egg in that half of the cases where the orientation is appropriate, or it might act as a second anaphase bridge which would probably be lethal to the zygote (STURTEVANT and BEADLE 1936; NOVITSKI 1951). If the former were the case, the ratio of crossovers to patroclinous males would be 3:3.

GENETIC RESULTS OF CROSSING OVER IN THE HETEROZYGOTES

The frequencies of recovered crossovers and patroclinous males in the experiments of L. V. MORGAN (1933) are 907 and 296, respectively. This is not in agreement with the expectation of 3 to 2 derived above. In order to distinguish crossovers in both sexes of the backcross generation, the parental males carried seven or eight mutant genes; some depression of the patroclinous male class might be expected as a consequence of the reduction of viability caused by these mutants. It will be shown below that this cannot account for the discrepancy.

This type of cross has been repeated. Females of the constitution X^{c2}, *cv v f/sc* were crossed to *sc cv v f B* males. The F_1 are given in Test A of table 1. The results are divided into two rows, with classes carrying recovered chromosomes of the same structure as the chromosome in the corresponding row of the genotype of the mother. In this case, the rods, identifiable by the marker *sc*, are given in the second row. The total number of crossover rods is 621; the number of patroclinous males is 313. The ratios of the two are calculated as 621 : 313 = 3 : X; X = 1.51. A comparison of the viability of males of the constitution *sc cv v f B* with sibs carrying no mutants suggests maximum depression of the viability of the order of 10 to 20 percent attributable to the multiple mutants. It should be noted also that the arrangement of the mutants in the parental females was deliberately designed so that, after crossing over, mutants would be removed from the ring chromosome and transferred to the rod, conferring a relative disadvantage to the recovered crossover rod chromosomes. It is clear, then, that the ratio of double crossover rod chromosomes to patroclinous males falls short of the calculated 3:2 ratio.

Other factors possibly affecting the expected ratio will not be considered in detail at this point; it may be pointed out, however, that any increment of patroclinous males originating in the E_2-3sb type of tetrad, or from primary nondisjunction, or any decrease in the observed crossovers by the occurrence of undetected doubles, expected to happen occasionally when mutants are as far apart as the ones used are, must lead to an error of opposite sign from the

TABLE 1

Genetic results from inversion heterozygotes giving rise to bridges of the various types. Symbols explained in text.

Test	Compositions of parental			Non- and double crossovers in regions							Totals		Ratio as 3:x
	Female		Male	0	1,4	2,4	3,4	1,3	2,3	1,2	co	Pat	
	Structural	Genetic											
A	$\frac{X^{c^2}}{N}$	$\frac{cv\ v\ f}{sc}$	sc cv v f B	4068 4504	19 76	38 159	17 47	47 137	56 194	4 8	621	313	3:1.51
B	$\frac{sc^8}{N}$	$\frac{sc^8}{sc\ cv\ v\ f\ B}$	w	1031 837	3 12	14 23	7 5	11 16	23 22	8 9	153	106	3:2.1
C	$\frac{sc^4}{N}$	$\frac{y\ sc^4}{sc\ cv\ v\ f}$	w^a	871 880	11 14	11 10	6 2	25 25	25 36	6 10	181	145	3:2.4
D	$\frac{YSX \cdot YL}{N}$	$\frac{y\ y^+}{sc\ cv\ v\ f\ B}$	w	919 808	16 19	23 19	11 7	27 44	23 25	9 12	235	88	3:1.1
E	$\frac{sc^8}{N \cdot YL(A\text{-}2)}$	$\frac{sc^8\ f\ v\ cv}{y\ w}$	w^a	1295 1716	13 9	9 15	6 7	22 18	14 14	1 1	129	41	3:1.0
F	$\frac{sc^8}{N \cdot YL(S)}$	$\frac{sc^8\ f\ v\ cv}{g\ B}$	w	1124 1405	5 6	9 20	4 22	20 8	17 1	5 9	126	62	3:1.5
G	$\frac{sc^8}{N \cdot YL(U\text{-}8e)}$	$\frac{sc^8\ f\ v\ cv}{y\ w}$	w^a	975 979	4 2	12 12	3 3	11 7	4 4	0 0	62	28	3:1.4
H	$\frac{sc^8 \cdot YL(P\text{-}8b)}{N}$	$\frac{f}{sc\ cv\ v}$	w	1247 1349	8 17	15 22	5 3	15 26	32 26	1 5	175	84	3:1.4
I	$\frac{sc^8 \cdot YL(K\text{-}7)}{N}$	$\frac{f}{sc\ cv\ v}$	w^a	1038 1042	11 18	14 22	9 5	26 32	19 36	6 9	207	85	3:1.2
J	$\frac{sc^8}{N \cdot YS(A\text{-}3)}$	$\frac{sc^8\ f\ v\ cv}{y\ w}$	w^a	1258 1740	7 9	5 10	2 4	19 9	14 14	5 2	100	65	3:2.0

TABLE 1 (*continued*)

Test	Compositions of parental			Non- and double crossovers in regions							Totals		Ratio as 3:x
	Female		Male	0	1,4	2,4	3,4	1,3	2,3	1,2	co	Pat	
	Structural	Genetic											
K	sc^8 / N·YS(M)	sc^8 f v cv / y w	w^a	1159 1585	4 3	4 15	1 4	7 12	10 10	0 0	70	53	3:2.3
L	sc^8 / N·YS(PDP)	sc^8 f v cv / y w	w	1069 1157	6 1	11 12	10 4	15 12	11 11	2 2	97	30	3:0.9
M	sc^8 / N·YS(U-8c)	sc^8 f v cv / y w	w^a	924 1126	3 8	9 15	5 7	10 16	16 16	2 3	105	46	3:1.3
N	sc^8·EN / N	y / sc cv v f	w^a,y^2w^a	8528 7256	72 64	105 87	22 23	113 130	123 117	32 30	918	623	3:2.0
O	sc^8 / N·YL(C-2)	sc^8f v cv / y w	y^2 w^a	1066 1107	1 1	6 3	1 0	14 15	11 11	0 1	64	7	3:0.3
P	YSX·YL / N·YL(A-2)	f v cv / y w	w^a	1067 1219	6 12	11 15	4 5	27 21	24 24	0 1	150	20	3:0.4
Q	YSX·YL / N·YL(U-8e)	f v cv / y w	w^a	892 1010	9 10	13 16	3 4	24 27	16 16	2 3	143	6	3:0.1
R	sc^8·YL(P-8b) / N·YL(A-2)	f / sc cv v	y^2 w^a	919 905	8 9	10 11	5 6	16 19	10 11	3 1	109	7	3:0.2
S	YSX·YL / N·YL(C-2)	f v cv / y w	w^a	752 875	11 7	7 9	1 2	20 20	20 20	4 6	127	5	3:0.1
T	YSX·YL / N·YS(PDP)	f v cv / y^2 w^a	w	864 1032	11 7	13 15	10 10	30 29	23 32	9 3	192	11	3:0.2

TABLE 1 (*continued*)

Test	Compositions of parental			Non- and double crossovers in regions							Totals		Ratio as 3:x
	Female		Male										
	Structural	Genetic		0	1,4	2,4	3,4	1,3	2,3	1,2	co	Pat	
U	YSX·YL / N·YS(U-8c)	f v cv / y w	w^a	799 876	11 6	7 8	2 2	16 18	13 13	3 3	102	7	3:0.2
V	sc^8·YL(K-7) / N·YL(A-2)	f / sc cv v	y w	1270 1169	13 12	15 16	7 2	37 22	21 28	5 2	180	21	3:0.4
W	YSX·YL / N·YS(A-3)	f v cv / y w	w^a	1165 1354	12 11	12 15	6 8	21 15	18 18	4 4	144	19	3:0.4
X	sc^8·YL(P-8b) / N·YS(A-3)	f / sc cv v	y^2 w^a	1082 1153	13 10	10 17	4 4	15 11	15 19	5 8	131	10	3:0.2
Y	EN·EN / N·YL(A-2)	f y / sc cv v	w^a, y^2, w^a	4556 4383	32 21	30 42	9 5	50 71	50 60	11 10	391	36	3:0.3
Z	EN·EN / N·YS(A-3)	f y / sc cv v	w^a, y^2 w^a	5272 5061	48 42	78 75	27 12	66 75	72 98	11 8	612	73	3:0.4

one under consideration. The possible influence of three exchange tetrads, or chromatid interference, either of which could effectively depress the frequency of patroclinous males, will be discussed after the description of experimental results with other types of material.

POSSIBLE EFFECT OF ASYMMETRICAL BRIDGES

From figure 1 it can be seen that the diagrammatic representation of the double chromatid bridge is asymmetrical, that is, at one end the centromere is terminal whereas the other end carries an entire X chromosome beyond the centromere. This is not the type of chromatid bridge upon which the assumptions concerning the behavior of double chromatid bridges have been based, for the earlier work (STURTEVANT and BEADLE 1936) involved bridges found by crossing over within heterozygous inversions of the X chromosome, both members of which had no appreciable chromatic material beyond the centromeres. Failure to account for the deficiency of patroclinous males in the experiments cited above as a consequence of any of the simpler variations in the tetrad analysis led to the assumption that the anomalous results might be a consequence of this asymmetry. This has turned out to be a very fruitful approach; the experiments to be described below involve the formation of such asymmetrical double chromatid bridges by four-strand double crossing over in heterozygous inversions, thereby eliminating any possible peculiarities inherent in the ring/rod type of heterozygote.

DESCRIPTIONS OF CHROMOSOMES USED

The mutants used throughout to determine the frequency of crossing over are *cv*, *v* and *f*, with a terminal marker at the locus of either yellow or scute. The regions given in table 1 are as follows: *y*(or *sc*)-*cv*, 1; *cv*-*v*, 2; *v*-*f*, 3; and, since only doubles or, very rarely, quadruples are recovered from exchanges occurring within an inversion, apparent singles must involve also an exchange between *f* and the centromere, region 4. In some instances other mutants were already present in the chromosomes tested; the regions, however, are listed in table 1 as the above and the few doubles found in such cases which would not be detectable without those mutants are not listed, in order to present comparable information from all tests. The omission of such doubles leads to an error that must be small but would, in any case, make the patroclinous males appear to be more frequent relative to the double crossovers. Females heterozygous for the mutants were outcrossed to males carrying a single mutant, or at most two. The inefficiency resulting from the limitation of the observed crossovers to the male sex only is outweighed by the advantage of having a patroclinous class which, if below expectation, cannot be viewed with suspicion from the standpoint of adverse viability effects.

Chromosomes of normal sequence used are of two types: those with the terminal (or, more precisely, subterminal) X centromere and those with an arm of the Y chromosome attached to form a double armed chromosome. The latter are all derived from detachments of attached-X chromosomes, of various

origins indicated by an experiment number in parentheses after the description of the extra arm. Of the inverted sequences, two are simple inversions without an extra arm, *sc*^{4} and *sc*^{8}. All others are derivatives of *In(1) EN,* a long inversion formed by the spontaneous opening-out of the ring X^{c}. This chromosome carries a short arm of unknown origin, known to be neither the long arm of the Y chromosome (YL) nor the short arm (YS) (NOVITSKI 1949). In the YSX·YL chromosome, this short arm has been replaced by the long arm of the Y from MULLER's *sc*^{8}·*Y* chromosome (1948) and YS has been attached to the tip of the X chromosome; resulting in a single chromosome carrying all the Y fertility factors and all the essential X chromosome genes (LINDSLEY and NOVITSKI 1950). The mutant *y,* present in the orginal *X*^{c} and located near the centromere in *In(1) EN,* is covered by the small duplication for the tip of the X on YL from MULLER's *sc*^{8}·*Y*. An inverted attached-X line was recovered from a stock carrying a chromosome possessing the base of the EN chromosome and the tip of *sc*^{8}; detachments of this *sc*^{8}*EN* attached-X provided chromosomes of inverted sequence with Y arms attached at the base. It should be noticed that such chromosomes are structurally different from *sc*^{8} only at the base. This chromosome has been described by LINDSLEY (1950); the writer wishes to thank him for the use of this stock, as well as for the detachment products extracted from it.

All detachments were tested by combining them with Y″ of STERN (1929) and Y^{cl} of MULLER (1948). Because Y″ lacks the male fertility factors on the long arm of the Y chromosome and Y^{cl} lacks those on the short arm, males carrying a detachment with the long arm of the Y attached to the base of the X are fertile with Y″ alone but not with Y^{cl} alone. Conversely, if a detachment-bearing male is fertile if it has also Y^{cl}, but not if it has Y″, it is concluded that the detachment carries the short arm of the Y chromosome. A few require further comment. Detachment *C-2* is lethal with Y^{cl}, although fertile with Y″. A female carrying this chromosome as well as one with *bb* shows a *bb* phenotype. There appears to be a heterochromatic deficiency at the base of the X chromosome involving at least two loci: (1) *bb;* (2) a region necessary for survival which is present in YS but not YL. Detachments *U-8c* and *U-8e* are members of a cluster recovered from a single female. Since they carry the two different Y arms, they are probably the complementary products of a single detachment crossover.

The compositions of the heterozygous females tested are given in table 1 in two columns, the first representing the structural arrangement of chromosome components and the second showing the arrangement of mutants on the two chromosomes. Above the line is given the inverted sequence of the X chromosome, followed by a dot to represent the centromere, and the composition of the second arm, when present, and finally, in parenthesis, a code identifying the culture in which that particular detachment occurred. The chromosome in normal order is shown below the line by the symbol N, followed by information similar to that above, when applicable.

DESCRIPTION OF THE PILOT EXPERIMENT

The tetrad analysis showing the consequences of crossing over within simple heterozygous inversions has been presented in detail by STURTEVANT and BEADLE (1936, 1939). The calculated ratio of double crossovers to nullo-X eggs from two exchange tetrads is 3 : 2; the ratio of crossovers detectable only in the male progeny to patroclinous males should also be 3 : 2. Their observed value of 3 : 1.95, as well as the earlier observations of STONE and THOMAS (1935) who arrive at a value of 3 : 2, is well in agreement with expectation. Tests B and C are two runs similar to the types described above. They give values of 3 : 2.1 and 3 : 2.4 respectively.

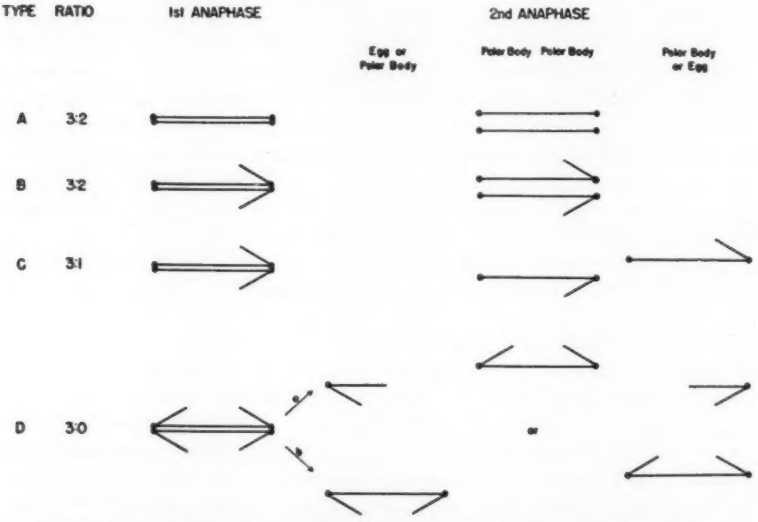

FIGURE 2.—The kinds of bridge configurations resulting from four-strand double crossing over in inversion heterozygotes, where the chromosomes involved may be characterized by an additional arm. The possible effect of dicentrics and chromatid fragments in decreasing the number of recoverable products from the four-strand double exchanges is illustrated as a consequence of (a) chromatid breakage and (b) centromere orientation.

Inversion heterozygotes made up of two chromosomes each with the nearly terminal centromere characteristic of the X chromosome produce by four-strand double exchange the type of double chromatid bridge given in line A of figure 2. However, when one of the chromosomes carries a Y arm at the base, an asymmetrical bridge of the type shown in lines B and C of figure 2 results. The results of the initial test of the assumption that asymmetrical bridges are responsible in some way for the depression of the patroclinous class are presented in line D of table 1. The ratio from this cross is 3 : 1.12, or just about half as many patroclinous males as expected. Tests E to O involve similar tests with the various combinations available to produce such

asymmetrical bridges. In all seven cases tested where YL is the arm at the base, the ratio is well below the expected 3:2. Two cases involving YS give about a 3:2 ratio (J, 3:2.0; K, 4:2.3) and two show the depressed ratio (L, 3:0.9; M, 3:1.3).

The extensive experiment (N) using the *EN* inversion gave a ratio of 3:2.0. Experiment O appears to be a special case, giving the very strongly depressed ratio of 3:0.3; a more detailed analysis of this detachment, *C-2*, will be presented below. A plot of the various ratios given in table 1 from tests B to N inclusive is strongly bimodal. Every value, in units of tenths, is present from 3:0.9 to 3:1.5. None occur between 3:1.6 and 3:2.0 and there are five values from 3:2.0 to 3:2.4 inclusive. The latter values will be generalized as the 3:2 type, the former as 3:1.

To account for this effect, it was hypothesized that the depression results from the lethality of half of the nullo-X eggs because of the inclusion within them of a dicentric chromosome. This is illustrated in figure 2. It can be visualized most simply as a consequence of unequal centromere "strengths" whereby both chromatids in the bridge are not kept out of the egg, but instead, a dicentric chromatid proceeds to one of the outermost products of meiosis which, in half the cases, would be the egg nucleus.

These possibilities are labelled in figure 2 in the following way: a bridge involving two normal X chromosomes is called type A; one formed by crossing over between a normal chromosome and one with an additional arm is called type B if the 3:2 ratio is maintained and type C if the ratio is shifted to 3:1. For brevity, chromosomes will be referred to as type B or C if they give type B or C bridges, respectively, when tested against a normal chromosome.

It is interesting to consider the possible consequences from a bridge when both chromosomes involved are of type C. The symmetrical bridges so produced (depicted in line D, figure 2) might be anticipated either to give again a 3:2 ratio, as the simple symmetrical bridge, or to eliminate the nullo-X class by breakage of the chromatid bridge and inclusion of a fractured chromatid into both distal products of meiosis.

Tests P to V inclusive show that the patroclinous male class is, in fact, almost completely eliminated and although the ratios are not 3:0 as it has been suggested they might be, they range from 3:0.5 to 3:0.1, and will be referred to collectively as the 3:0 type. The simple concept of centromere strengths, adequate to account for the above results, is not sufficient to account for those from other types of tests. In every case where both termini of the bridge carry heterochromatic arms the ratio is the 3:0 type. This includes bridges characterized by a type B chromosome at one end and type C at the other (experiments W, X, and Y) and, most curiously, the combination where two type B chromosomes are involved (experiment Z).

Not included in table 1 are the results from seven other tests. Six are the combinations of $sc^8 \cdot YL(K\text{-}7), f\,v\,cv$ and $sc^8 \cdot YL(P\text{-}8b), f\,v\,cv$ each with $N \cdot YL(U\text{-}8e), y\,w$, with $N \cdot YL(C\text{-}2), y\,w$ and with $N \cdot YS(PDP), y^2\,w^a$.

The seventh was a test of $sc^8 \cdot YL(K\text{-}7), f\, v\, cv$ with $N \cdot YS(U\text{-}8c), y\, w$. Crossovers were not classified individually; only the total males and patroclinous males were noted. From table 1 it can be shown that the ratio of patroclinous males to all males is 0.037 in those cases giving a 3:2 ratio, 0.024 in type C and 0.005 in type D. The individual values in the above tests ranged from 0.009 to 0.003 with an overall ratio of 93/17365 or 0.005. This is considered adequate evidence that these cases likewise are of the 3:0 type.

FURTHER ANALYSIS OF THE C-2 DETACHMENT

The one discrepant case in table 1 is that of test O, where an asymmetrical bridge produced by crossing over between the sc^8 chromosome and the *C-2* detachment gave a depressed frequency of patroclinous males. Such a result could be expected if one of the chromosomes carried a " weakpoint " such that the double bridges always broke and fractured chromatids were delivered to the terminal products of meiosis. In order to be included as part of the dicentric and not eliminated in the acentric fragments, such a weakpoint would have to be located near the base of one of the participating chromosomes, in this case *C-2*, since other tests with the specific sc^8 chromosome used here showed no evidence of this effect.

The localization of the factor responsible for the depression was carried out in the following way. Males of the constitution $N \cdot YL(C\text{-}2), y\, w/Y''$ were mated to *sc cv v f* females. F_1 females were mated to $sc^8\, f\, v\, cv$ males. Certain crossover classes were identifiable in the next generation; these, as well as some noncrossovers, were crossed to YSX · YL, *y B* males. The types of progeny from this cross showed more clearly the specific nature of the crossover chromosome being tested. Since all (except the patroclinous) male progeny from this kind of mating are XO, identification of the lethal effect at the *C-2* base was provided by the appearance of sc^8 males only. The various crossovers differed, of course, in their genetic make-up so that the usual crossover counts could not be made, and the low viability of sc^8 in the XO male made unreliable the measure provided by the ratio of patroclinous males to the total. Instead, the behavior is recorded as the number of patroclinous males per bottle. Since individual females were tested concurrently under moderately uniform conditions, this criterion would appear to be reliable.

It is seen from table 2 that the number of patroclinous males per test is 1.7 or below in every case where the chromosome tested carries the lethal at the base of the *C-2* chromosome, and it is 2.0 or above in those cases where the lethal is not present, a sole exception being the 2,4 double class which gave 2 patroclinous males in 3 tests. It would appear, therefore, that the effect of decreasing the relative frequency of patroclinous males is brought about by some condition near the base of the *C-2* chromosome. It has been pointed out that there is evidence for a heterochromatic deficiency at the base; that this is not responsible, however, is shown by the following result. Females of the constitution *sc cv v f* were mated to $y\, w \cdot YL(C\text{-}2)$ males. F_1 females were mated to $g\, B \cdot YL/Y''$ males, and *sc cv v f* males were selected from their progeny.

TABLE 2

The behavior of chromosomes resulting from crossing over between sc cv v f and y w (1)· YL(C-2) when tested with sc^8 / v cv, showing the type of crossover, the genetic constitution of the tested chromosome, with the lethal from the C-2 chromosome indicated by (1) when present, and the number of patroclinous males per tested chromosome.

Crossover region	Constitution of chromosome	Numbers of		Pat. males per test
		Pat. males	Tests	
0	y w (1)	11	15	0.7
0	sc cv v f	31	13	2.4
1	sc (1)	3	5	0.6
1	y w cv v f	22	7	3.1
2	sc cv (1)	8	11	0.7
2	y w v f	35	13	2.9
3	sc cv v (1)	7	11	0.6
3	y w f	24	10	2.4
4	y w	6	3	2.0
1,2	sc v f	2	1	2.0
1,3	y w cv v (1)	0	3	0.0
1,3	sc f	7	2	3.5
1,4	sc	5	1	5.0
2,3	y w v (1)	5	3	1.7
2,3	sc cv f	4	2	2.0
2,4	sc cv	2	3	0.6

Such males should be sterile unless a crossover beyond forked gave a chromosome of the composition *sc cv v f· YL*. A stock was established of this type; this chromosome was then placed against sc^8 and mated to $y\,w\cdot YS(U\text{-}8c)/Y^{cl}$ males. The ratio from this cross was 376 : 133 or 3 : 1.1. The lethal effect was still present at the base which carried YL and gave the ratio characteristic of all chromosomes tested carrying YL. It would appear, then, that the region responsible for this effect, or, in terms of the breakage idea, the weakpoint, is located between the centromere and forked, but to the left of the loci involved in the lethal effect of the *C-2* chromosome.

THE ISOLATION OF BREAKAGE PRODUCTS

Analysis of the types of chromosome rearrangements produced after irradiation has led to the conclusion that, in Drosophila at least, simple chromosome breaks do not heal (Muller, 1940). It is not known whether, in Drosophila, the bridges produced by crossing over within ordinary inversion heterozygotes ever break, but it seems likely that they do not since there does not appear to be any zygote mortality that can be attributed to this cause in experiments with heterozygous inversions. In the experiments being described here, however, there is a deficiency of from 50 to almost 100 percent of the class of four strand doubles and, as pointed out above, such mortality might be the result of the inclusion of a fractured chromatid in the functional egg nucleus. Now if in some small proportion of the cases where the egg received a fractured chromatid the broken ends were to heal, the occasional recovery of simple breaks might be considered evidence that the deficiency in the nullo-X class

is in fact a consequence of the inclusion of broken chromatids in the functional egg nucleus. A recoverable broken chromatid would necessarily be either very short, to survive in the male as a duplication, or very long, to survive in the female as a deficiency. Breaks in the heterochromatic regions, if they occurred and healed, would therefore be more amenable to detection than euchromatic breaks.

Figure 3 shows the method used to detect the products of breakage. As pointed out above, the YSX · YL chromosome carries a recessive yellow near the centromere, and the normal allele at the tip of the long arm of the Y attached at the base. A crossover between it and a chromatid in normal sequence gives rise to a bridge. (Only two chromatids are drawn in figure 3; another set of two would be similarly involved in the four-strand double class.)

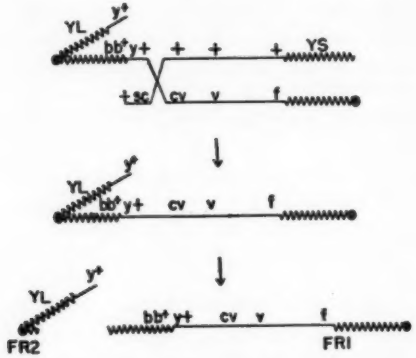

FIGURE 3.—The type of configuration and genetic constitution of the bridge type which produced recoverable fragments. The distribution of mutents, crossover region, and point of breakage, are the same as those in the actual experiment. The figure does not include the sister chromatids of those shown, which also must have undergone exchange if the fragments originated in the four-strand double class.

If the bridge should break at any point in the heterochromatic region between *y* and the centromere, with a stabilization of the broken end, two fragments would result. The first would be a chromatid of normal sequence, with *y* and some heterochromatin at the tip, and with or without the other mutants depending upon the position of the crossover forming the bridge. The other fragment would carry the normal allele of yellow on the long arm of the Y chromosome.

Females heterozygous for the YSX · YL chromosome and a normal chromosome carrying *sc cv v f*, as diagrammed in figure 3, were mated to YSX·YL, *y B* males. The use of the *YSX · YL* chromosome makes it possible to isolate the smaller breakage product in a fertile F_1 male. From this mating there were 3573 non-yellow females, 3589 non-yellow Bar males, 191 yellow Bar males (patroclinous), one yellow Bar female, one Bar male and one Bar male mosaic for yellow.

Two other types were examined for breakage products. One involved females of the constitution $YSX \cdot YL/y^2\, cv\, v \cdot YS(PDP)$ and the other $sc^8/sc\, cv\, v\, f \cdot YL(C\text{-}2)$, both mated to $YSX \cdot YL, y\, B$ males. From the first were counted 4441 non-yellow (but yellow^2) females, 5950 non-yellow non-Bar males, 36 yellow Bar (patroclinous) males; from the second, only the longer type of fragment could have been recognized as a yellow Bar female. Of 15080 F_1 females, none were yellow.

The three exceptional individuals noted above have been analyzed further. The mosaic was fertile, but no non-yellow progeny appeared in a mating to a yellow female. The yellow Bar female proved to carry a chromosome which was *y cv v f* and was apparently inverted with respect to the YSX · YL chromosome. Females heterozygous for this chromosome, named FR1, and $y\, w \cdot YL(C\text{-}2)$ were mated to yellow males carrying the YSX · YL so that all the F_1 males would be XO. Single crossover progeny with the base of the C-2 chromosome and the tip of FR1 were viable; that is, attached to the end of FR1 was a section of heterochromatin covering the lethal locus of the C-2 chromosome. A similar test with a chromosome carrying *bb* gave F_1 males that were never *bb* when they were *y*, providing additional evidence for the presence of a heterochromatic section remaining after breakage. The simplest interpretation of the origin of this chromosome is that it resulted from the breakage of a chromatid bridge.

A simple crossover test of $y\, cv\, v\, f\ (FR1)/y^2$ gave the following crossover values: *y-cv*, 19/414 (4.6 percent); *cv-v*, 54/414 (13 percent); *v-f*, 84/414 (20 percent). The normally expected values are about 13, 20 and 20 percent, respectively. It appears that the presence of the distal heterochromatin has the effect of suppressing crossing over near it, with the effect decreasing as the crossover becomes more distant from it.

The second fragment, FR2, which carried the normal allele of yellow as a small duplication, was shown to contain all the fertility factors of YL, by combining it with a detachment carrying YS. However, it should be pointed out that there is in this case another possible origin. Crossing over between YL of the YSX · YL chromosome and the heterochromatin at the base of the normal chromosome would yield such a free fragment. This fragment would have the terminal centromere of the normal X, whereas the breakage product could involve a small section beyond the centromere depending upon the position of the break in the bridge. In somatic mitoses the centromere appears terminal. It is not possible therefore to distinguish between these alternatives.

Although the other two tests did not yield any types identifiable as breakage products, the cross of $sc^8/sc\, cv\, v\, f \cdot YL(C\text{-}2) \times YSX \cdot YL, y\, B$ produced two fertile matroclinous (non-Bar) females. Cytological and genetic observations indicate that they carried a compound X chromosome, with the two X's hooked end to end, but in reverse order of each other. They are structurally similar to the double X derived by MULLER (1943), except that they do not have an interstitial inversion, as the latter does, thereby making the chromosome potentially useful for crossover studies.

DISCUSSION

The results from inversion heterozygotes necessitates a re-examination of the tetrad analysis for ring/rod heterozygotes. The ratio of 3 : 1.5 arrived at during the course of the above experiments appears to the writer to be fairly reliable; it is probably not a variation, of either statistical or experimental origin, from either a 3 : 1 or a 3 : 2 ratio. It has been noted that the tricentric type of chromatid bridge might produce nullo-X eggs; if so, the ratio of double crossovers to patroclinous males would be 3 : 3. A correction factor of one half for the asymmetrical bridge effect would give the 3 : 1.5 observed. While there is reason to believe that the correction of 50 percent might be appropriate for the four-strand double class, which gives asymmetrical bridges, a similar correction for the tricentrics is not justifiable on the basis of the limited knowledge of such cases.

Two factors dismissed earlier as probably not being responsible for the depression of the ratio in the case of ring/rod heterozygotes may be more effectively eliminated using the extensive data, with more extreme effects, from the inversion heterozygotes. From three exchange tetrads a ratio of double crossovers to patroclinous males of 21 : 4 is expected (STURTEVANT and BEADLE 1936). An appreciable number of such tetrads would cause the patroclinous male class to be depressed in the same direction as that observed. If this were responsible, the observed rate of crossing over should be higher from tests giving the lowest ratios. The rate, measured by the number of crossovers divided by the number of noncrossovers, for the three cases, is as follows: 3 : 2, 1922/25145 = 5.7 percent; 3 : 1, 1136/18173 = 6.3 percent; and 3 : 0, 2345/39848 = 5.9 percent. These are not statistically different.

The uniform overall rates of crossing over between the sets likewise argues against the application of the concept of chromatid interference. One of every three recovered double crossover strands originates in a two-strand double tetrad, the other two originate in a three-strand double. Chromatid interference would have to act exclusively upon the four-strand double class, or also upon the two- and three-strand double class in a complementary fashion. It cannot be denied conclusively that the deviation from expectation of the results from ring/rod heterozygotes is an indication of chromatid interferences, especially since it is in heterozygotes characterized by this sort of structural complexity that chromatid interference might be expected to become most obvious, if it exists at all in Drosophila. However, the duplication and even exaggeration of the effect using specified types of rod chromosomes for which there is no independent evidence suggesting chromatid interference is a strong argument against it.

The possibility that products of crossing over between heterozygous inversions might give rise to a lethal class by their inclusion in the egg nucleus has been considered in detail by all the earlier workers in this field; the concepts developed by STURTEVANT and BEADLE resolved the perplexing question of why there was, in fact, no appreciable mortality from single chromatid bridges.

The data presented above do not conflict with their conclusions on the behavior of such bridges, but suggest that the double chromatid bridges of certain structural configurations do not give the anticipated effects. It may be remarked, in this connection, that the double bridge types produced by the detachments may not represent a special case, but that, on the contrary, the normal X chromosome of *D. melanogaster* with its almost terminal centromere may be exceptional. There appears to be an excellent likelihood that the autosomes would behave as the 3:0 rather than the 3:2 type if one should develop methods of testing their behavior.

Several arguments may be applied against considering the acentric fragments generated by crossing over within inversion heterozygotes as a possible source of lethality of the nullo-X class. In the first place, the ring/rod heterozygotes, which first indicated the incompatibility between expectation and observed, do not produce acentric fragments by crossing over. Secondly, the acentric fragment is composed of the two ends of the chromosome joined together. From table 1 it can be seen that different chromosome combinations producing identical types of acentric fragments give different ratios. Finally, the type of behavior of a chromosome depends upon the nature of its base. The addition of large sections of a normal chromosome to a detached type has never changed the pattern of effects characteristic of the detached type so long as the base itself is not removed. The C-2 detachment would appear to be an exception to this, but actually is not, for when the region close to the base apparently responsible for its unique effects when with a normal chromosome is removed by crossing over with a normal chromosome, it then behaves similarly to all other detachments carrying the long arm of the Y chromosome.

Lethality in the four-strand double class might result either from the inclusion of a dicentric, or from a breakage product of a dicentric. These two possibilities are illustrated in figure 2. When the frequency of patroclinous males is halved, as it is in type C, then it appears more likely that the dicentric would be responsible for death than a fragment, for if the bridge broke under these conditions, each product of the first division would receive two fragmented chromatids. Consequently both terminal nuclei would receive defective chromatids with an expectation of 3:0 rather than 3:1. In the case of type D, however, the possibilities are more ambiguous. Either the breakage of a chromatid bridge (or both bridges) followed by the inclusion of a fragment into both terminal nuclei (possibility a) or the regular separation of the two dicentrics from each other, each into a terminal nucleus, (possibility b) would be consistent with the results.

The alternative, that a dicentric is always (or almost always) directed into the egg nucleus in the 3:0 type, and in half the cases in the 3:1 type, may be visualized as a consequence of an orientation imposed on sister centromeres by the adjacent heterochromatin. This possibility may be more readily appreciated if the various ways in which sister centromeres may separate at the second meiotic division are considered in detail. After the first division, both sets

of unsplit centromeres are bound together in the double dicentric. If both terminal centromeres of a given dicentric chromatid tend to separate from their respective sister centromeres in opposite directions, one of the pair of dicentrics would collapse; in neither case would a dicentric move to an outer nucleus. However, if both terminal centromeres of a dicentric chromatid moved in the same direction at second anaphase, the movement of that dicentric would be toward an outer nucleus and since, presumably, sister centromeres always move away from each other, the other dicentric of the double bridge would move towards the other outer nucleus. After the second division, the positioning of the dicentrics might be similar to those of second anaphase bridges which produce lethal zygotes. In the first case, the genetic result would be of the 3 : 2 type; in the second, the 3 : 0 type. The 3 : 1 type might be explained as a random mixture of the two types above in which the sister centromeres at one end of the bridge separate in a direction independent of separation at the other end. The direction of separation of both sets of sister centromeres could be random, or one set could have a fixed orientation, with the opposing set at random; either type would produce a 3 : 1 ratio. The latter, however, is more consistent with the observations.

Like the alternative of centromere strengths, the idea that such an orientation of the sister centromeres imposed somehow by the adjacent heterochromatin is responsible for the depression of the nullo-X class does not offer any simple explanation of the 3 : 0 type ratio always obtained when a bridge has both termini characterized by extra arms. These two possibilities might be differentiated cytologically, but this approach appears to be impracticable for technical reasons. The genetic analysis appears at present to be limited by the kinds of material available and it seems likely that the further resolution of the problem will depend upon the development of chromosome types not now in existence.

SUMMARY

It has been shown by STURTEVANT and BEADLE that dicentric chromatids produced at meiosis by crossing over within heterozygous inversions in Drosophila do not ordinarily reach the egg nucleus because the chromatid bridges thus formed center the dicentrics in the two innermost nuclei of the four meiotic products. When two crossovers involving all four chromatids (four-strand double) produce two such dicentrics, the outer nucleus destined to become the egg nucleus receives no chromatid from the tetrad. It is possible to detect such occurrences when the X chromosome is under observation because nullo-X eggs give rise to patroclinous males when fertilized by X-bearing sperm. Furthermore, the frequency of this class bears a simple numerical relationship to the frequency of observed double crossovers, which come predominantly from two-strand and three-strand double exchanges.

Experimental results show that the above formulation holds only when the chromosomes involved are essentially telocentric, or, in some cases, when one, but only one, of the two chromosomes carries a small short arm not involved

in the bridges. In some cases where the additional arm is small (as the short arm of the Y chromosome) and in all cases where it is longer (the long arm of the Y chromosome), the observed frequency of patroclinous males is about half that expected. In every case tested where both X chromosomes are characterized by the presence of additional arms, the patroclinous male class is eliminated almost entirely.

Alternative interpretations based on bridge breakage and centromere orientation are discussed in detail. In either case, the deficiency of patroclinous males is considered to be a consequence of the delivery to the egg nucleus of a structurally aberrant chromatid, a fractured chromatid under the first interpretation and a dicentric chromatid under the second. Those aspects of the experimental results bearing on the differentiation of these interpretations are considered at length.

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INHERITANCE IN NICOTIANA TABACUM. XXIV. INTRASPECIFIC DIFFERENCES IN CHROMOSOME STRUCTURE

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THE tobacco of commerce, *Nicotiana tabacum,* is a cultivated tetraploid species which has arisen by hybridization between two diploid species followed by doubling of the chromosome number. When, however, the amphidiploid involving its putative parent species was experimentally produced it was found that certain features were markedly different from the derived species, notably its complete female sterility. It is, therefore, of considerable theoretical and practical interest to determine the nature of the changes which have occurred since the original allopolyploids appeared. One avenue of approach to the problem is a study of the differences in chromosome structure among the numerous varieties. These include a number of more or less improved widely grown commercial types as well as representatives of relatively small local populations, door-yard varieties, etc., the majority of which have been collected in the Andean region of South America. So far as is known there are no wild representatives of the species in the strict sense. Cytological examination reveals the presence of 24 bivalents in all of these varieties, but when they are hybridized, the F_1 hybrids in many instances exhibit associations of four and six chromosomes, indicative of the induction and establishment of reciprocal translocations. MALLAH (1943) first demonstrated and analyzed a few such differences. Since that time many additional varieties have been studied and the identity of most of the translocated chromosomes has been determined.

MATERIALS

In addition to the 15 varieties studied by MALLAH, 40 more recent accessions have been examined in hybrid combination. In general, 26 are of the familiar commercial type with large leaves, rather coarse central stems and terminal inflorescences. One of them, the highly inbred variety Purpurea, has been used as a standard of reference throughout the investigation. The other 29 varieties are more weedy in habit and are characterized by the production of relatively small seeds. The latter characteristic has proved of value in selecting varieties to be examined for possible chromosome interchanges. Cytological studies were made on temporary preparations of hybrid anthers stained with aceto-orcein as outlined by CLAUSEN and CAMERON (1944).

VARIETAL CLASSIFICATION ON THE BASIS OF CHROMOSOME HOMOLOGIES

Each of the varieties included in the following account has been crossed with variety Purpurea and the resulting hybrids subjected to cytological study.

Whereas translocation complexes are not the only evidences of difference these are the only ones which appear to be amenable to analysis. A decrease in the normal chiasma frequency is indicated in some hybrids and in at least one a heteromorphic bivalent may be recognized in most cells. Anaphase bridges with accompanying fragments suggest the presence of inverted segments in some hybrids.

In table 1 the chromosomal associations of representative varieties in relation to certain selected testers are summarized. Those which exhibit complete bivalent formation in hybrids with Purpurea have not been tested further. All the varieties with relatively interchanged chromosome segments have been studied in hybrid combination with Huadquina and Apolo Red; also Chinchao and/or Cuba White. Eight varieties are similar to Huadquina in their pairing relationships and 11 are included with Coripata for the same reason. The latter group requires further explanation. Its members regularly give rise to

TABLE 1

Chromosomal association in varietal hybrids.

	Purpurea	Huad.	Apolo R.	Chinch.	Cuba W.
Coripata	4	4(?)	4+4	4+4	....
Ceniza	4	4+4	6	6+6	4+4+6
Cerro Alegre	4+4	4	4+6	4+4+4	4+4+4
Pabur	6	4	4+6	4+4+4	4+6
Cuba White	4+6	4+4	4+4+6	4+4+4+4	
Chinchao	4+6	4+4	6+6		
Apolo Red	4	4+4			
Huadquina	4				

a ring of four in hybrids with Purpurea. However, in combination with Huadquina chromosomes the cytological results are inconsistent. Some of these hybrids have a fairly high frequency of cells with a ring of four, indicating that the same chromosomes are involved but with a different segmental arrangement. In others a difference in structure is revealed only by a failure of complete bivalent formation ($2_{3II}+2_I$). If an association of four were regularly present in Coripata–Huadquina hybrids, then the combination Coripata–Chinchao should have two complexes of four and six, but in each case the same result is obtained as that in F_1 Huadquina–Chinchao (4+4). Further studies on this group are under way.

The varieties which have been investigated are listed below with their places of origin as nearly as it is possible to determine them. The varietal names have no special significance and are used merely for convenience. For more specific identification, accession numbers are included wherever such numbers have been assigned and in most cases these are the UNIVERSITY OF CALIFORNIA BOTANICAL GARDEN numbers. The varieties have been listed in groups corresponding to their above-described cytological relationships.

LARGE SEEDED VARIETIES

Variety	*Accession no.*	*Source*	*Obtained from*
1. Purpurea group			
Ambalema	37-51	Columbia	J. A. Nolla
Apolo Pink	39-246	Bolivia	T. H. Goodspeed
Belge	40-25	Canada	N. A. MacRae
Bergerac catacorolla	40-29	France	H. P. Olmo
Brabazon		Iran	H. P. O.
British Guiana	44-2	Br. Guiana	O. Goodson*
Charpan		Iran	H. P. O.
Colombian M. R.		Colombia	E. E. Clayton
Consolation	40-122	Puerto Rico	J. A. N.
Corrientino 39		Paraguay	Compania Chilena de Tabacos
Corrientino 57		Chile	C. C. de T.
Corrientino 59		Chile	C. C. de T.
Corrientino 60		Chile	C. C. de T.
Criollo palo Amarillo		Argentina	C. C. de T.
Daruma		U. S.	J. Johnson
Girishk		Afghanistan	H. P. O.
Habano Chagres		Paraguay	C. C. de T.
Laguna Sielo	39-32	Argentina	Eyerdam and Beetle*
Maryland mammoth	31g1	U. S.	E. G. Beinhart*
Papago	39-286	U. S.	T. H. G.
Plomo		Chile	C. C. de T.
Purpurea	06-25	U. S.	Missouri Botanic Garden
San Felipe		Chile	C. C. de T.
Serrate	40-23	U. S.	E. E. C.
Station Standup	40-26	Canada	N. A. MacR.
Trelease Turkish	40-24	U. S.	S. F. Trelease

SMALL SEEDED VARIETIES

Variety	*Accession no.*	*Source*	*Obtained from*
2. Apolo Red	39-245	Bolivia	T. H. G.
3. Ceniza	40-27	Colombia	J. A. N.
4. Cerro Alegre	39-146	Chile	T. H. G.
5. Pabur	39-213	Peru, dept. Piura	T. H. G.
6. Chinchao	36-38	Peru, dept. Huanuco	T. H. G.
7. Cuba White	14-200	Unknown	Mrs. Rose Thomas*
8. Huadquina group			
Amazonas	42-31	Peru, dept. Amazonas	R. D. Metcalf*
Cachicadan	40-42	Peru, dept. Libertad	T. H. G.
Canchaque	39-125	Peru, dept. Piura	T. H. G.
Chachapoyas	42-29	Peru, dept. Amazonas	R. D. M.*
Coquimbo	36-49	Chile	T. H. G.
Cuzco	36-14	Peru, dept. Cuzco	Y. Mexia*
Huadquina	36-170	Peru, dept. Cuzco	T. H. G.

SMALL SEEDED VARIETIES (*continued*)

Variety	*Accession no.*	*Source*	*Obtained from*
Lima	43-29	Peru, dept. Lima	T. H. G.
Sartimbamba	44-4	Peru, dept. Libertad	S. Gastanadui
9. Coripata group			
Acomayo Red	39-184	Peru, dept. Cuzco	T. H. G.
Alameda Red	39-290	U. S.	T. H. G.
Cajamarquilla	45-1	Peru, dept. Libertad	S. G.
Chulque Pink	36-39	Peru, dept. Huanuco	T. H. G.
Chulque Tinged	36-38	Peru, dept. Huanuco	T. H. G.
Coripata	46-48	Bolivia	T. H. G.
Coroica	36-43	Bolivia	T. H. G.
Huanuco	36-58	Peru, dept. Huanuco	T. H. G.
Huaras	45-2	Peru, dept. Huaras	N. van der Walle*
Leimebamba	42-43	Peru, dept. Amazonas	R. D. M.*
Marcapata	39-182	Peru, dept. Cuzco	T. H. G.
Mirador	36-47	Peru, dept. Huanuco	T. H. G.
10. Sandia	42-19	Peru, dept. Puno	R. D. M.*
11. Trujillo	37-4	Peru, dept. Libertad	T. H. G.

*By courtesy of Prof. T. H. GOODSPEED.

MALLAH has already shown that the complexes of Huadquina and Apolo Red are independent and that those of Ceniza and Apolo Red include a common chromosome. In addition, table 1 shows that the same chromosome is present in both the Cerro Alegre and Apolo Red complexes. The other Cerro Alegre translocation is presumably identical or very similar to that of Huadquina. The complex of six in Pabur evidently includes the same translocation that is present in Huadquina. A similar situation was observed in the earlier study with respect to the Huadquina–Cuba White hybrid. Cytological studies of the various hybrids also show that while Chinchao and Cuba White are both characterized by the presence of three translocations involving five chromosomes, some of the chromosomes affected must be different. Two other varieties have been studied incompletely. One, Sandia, has the largest number of translocated chromosomes thus far uncovered, giving three complexes (4+4+6) in hybrids with Purpurea. However, not enough is yet known to place them in their proper relationship to the other varieties.

TYPES OF ASSOCIATION IN THE 4-CHROMOSOME COMPLEXES

Frequencies of different types of association in the 4-chromosome complexes are shown in table 2. In most of these the translocated segments are

TABLE 2

Types of configurations in 4-chromosome complexes in F_1 hybrids involving Purpurea.

	Ring of four	Chain of four	Trivalent and single	Two bivalents	One bivalent two singles	Four singles	Total
Huadquina	112	24	9	5	2	1	153
Apolo Red	203	32	1	9	6	1	252
Chinchao	140	69	12	24	5	1	251
Cuba White	135	26	2	1	1	....	165
Ceniza	9	52	1	91	5	1	159
Cerro Alegre	248/2*	32/2	3/2	24/2	3/2	....	155

*Two indistinguishable associations of four chromosomes are present in each cell.

apparently of appreciable length, enabling the chromosome arms to form the minimum number of chiasmata required for ring formation. The exception, Ceniza, produces bivalents in the majority of hybrid cells. This variety has the same altered chromosome as Apolo Red and Chinchao, but apparently the breaks have occurred in different regions. The last two classifications are, of course, presumptive as the observed univalents may be the result of chiasma failure in normal bivalents. While this material does not lend itself to a study of chromosome orientation within the rings, it can usually be observed in polar views that the opposed chromosomes lie at a different level of focus. Thus, it seems likely that alternate distributions predominate. This, in combination with the polyploid nature of the species, leads to a very high degree of pollen fertility. Even in heterozygotes with two rings (4+6) the percentage of stainable pollen is well within the normal range. *Datura stramonium* (BERGNER *et al.* 1933) and *Triticum monococcum* (THOMPSON and THOMPSON 1937) also may have little pollen abortion in translocation heterozygotes even without the duplication of material afforded by the allopolyploid condition. SNYDER (1951) reports considerable variation in pollen fertility in translocation heterozygotes in *Elymus glaucus*.

IDENTIFICATION OF THE INTERCHANGED CHROMOSOMES

The monosomics of variety Purpurea (CLAUSEN and CAMERON 1944) provide a simple though laborious method of identifying the particular chromosomes which take part in the characteristic complexes of the groups delimited above. This was accomplished by crossing selected monosomic plants from the complete series with the following varieties: Apolo Red, Ceniza, Huadquina, Cerro Alegre, Pabur, Chinchao and Cuba White. The resulting populations consist of normal hybrids which show the cytological situation characteristic of the original Purpurea hybrids and 47-chromosome plants lacking a normal Purpurea chromosome corresponding to the particular monosomic used. If this chromosome is ordinarily a component in a ring of four the constitution of the hybrid will be 22 bivalents plus a chain trivalent. The latter unit will comprise a normal chromosome attached at either end to modified ones. Thus,

in the hybrid Purpurea–Huadquina the monosomic plants of populations haplo-E and haplo-K give rise to chain trivalents while the $2n-1$ plants of the other 22 cultures have cells containing $21_{II}+4+1_{I}$. In the haplo-E culture this trivalent is made up of a normal K chromosome and two translocation products involving E and K chromosomes. Purpurea monosomic types haplo-J and haplo-L were isolated by MALLAH (1942) as products of asymmetric distribution from the Cuba White ring of four and the composition of this complex was verified by the present technique. Table 3 shows the make-up of the other complexes which have been investigated. So far the third member of the Chinchao 6-complex has eluded identification and the assignment of the D chromosome to the Pabur association necessarily remains tentative. Further consideration of these features will be reserved for the discussion.

CYTOLOGY OF BACKCROSSES

As we have seen, the chain trivalent present in $2n-1$ varietal hybrids is presumably made up of a Purpurea chromosome associated with two relatively interchanged units. According to expectation, duplication-deficiency gametophytes are nonfunctional and 23-chromosome pollen grains rarely effect fertilization (OLMO 1935). As a result, backcrosses resulting from monosomic Purpurea pollinated by corresponding $2n-1$ hybrids should include only two types. Fertilization of 24-chromosome egg cells should produce plants with meiotic configurations of $22_{II}+4$ while those developing from 23-chromosome gametophytes should again contain the trivalent.

A study of 32 plants from backcrosses involving the Purpurea–Huadquina hybrid shows that both interchange chromosomes were present in all fertilizations resulting in disomic plants. However, among the monosomic progenies the results differed depending on which monosomic type was used as pollen parent. Haplo-K Purpurea–Huadquina individuals gave rise to meiotic configurations of $22_{II}+3$ in all backcross plants studied. Such backcrosses involved both haplo-E and haplo-K Purpurea as ovule parents. When haplo-E Purpurea–Huadquina plants were used as pollen parents about half the $2n-1$ individuals studied formed $22_{II}+3$, and the remainder $23_{II}+1_{I}$. This may mean that haplo-E is capable of relatively high transmission through the pollen. However, in no instance where haplo-K Purpurea was pollinated by haplo-E Purpurea–Huadquina were the derived monosomic plants classed as

TABLE 3

Identification of the interchanged chromosomes.

	"tomentosa"							"sylvestris"				
Apolo Red									Q		S	
Ceniza											S	Z
Huadquina		E		K								
Cerro Alegre		E		K				P	Q			
Pabur	(D)	E		K								
Chinchao		E		K		?	N				S	
Cuba White		E	J	K	L					R		

haplo-E on the basis of morphological criteria. The size of the univalent chromosome in cells where the trivalent was replaced by a bivalent and univalent also indicated that the 23-chromosome gamete was contributed by the female parent.

Backcross populations involving hybrids characterized by two associations ($19_{II}+4+6$) were also produced. Depending on the particular monosomic used as ovule parent it would be expected that the ring of four or six would be replaced by a trivalent or chain of five, respectively, in appropriate crosses. *A priori,* one would expect the remaining association to be replaced by bivalents in about half the individuals since gametes with " standard " chromosomes as well as those with " interchanged " ones would be equally viable. This has only been observed in two of 23 such backcross individuals studied. Apparently selective influences favor the retention of the heterozygous condition. This feature should be followed up using larger numbers and reciprocal combinations.

DISCUSSION

Many of the genetic features of *N. tabacum* are known to be governed by duplicate genes (CLAUSEN and CAMERON 1944, 1950) and in each case one has been located in the " sylvestris " and one in the " tomentosa " genome. Also, cytological studies of the diploid hybrid involving these species have revealed a small amount of loose chromosome pairing ($0–7_{II}$). Thus, it might be expected that reciprocal translocations would arise by some process analogous to crossing over in small segments of otherwise nonhomologous chromosomes in the two sets. The present study, however, reveals that in each ring of four investigated thus far, both altered chromosomes are members of the same genome, chromosomes A-L representing the tomentosa contribution. Four of the rings are composed of sylvestris chromosomes (N-S, P-Q, Q-S, S-Z) and two include chromosomes from tomentosa (E-K, J-L). The 6-complex of Cuba White is unique in this respect, having chromosomes from both genomes (E-K-R).

Since the E-K translocation appears in six of 11 associations that have been investigated, it is conceivable that in reality, these two chromosomes have become modified in the line which we have used as standard and in those related most closely to it. Suggestive evidence is provided by the fact that these two chromosomes are at the extremes of size variability within the variety Purpurea. Also it is known that a single ring of four is formed in hybrids between this variety and the experimentally produced amphidiploid, 4n-sylvestris-tomentosa. It will be of interest to examine the hybrids between this amphidiploid and other varieties such as Huadquina or some member of the Coripata group.

Assuming that the Purpurea group is characterized by the presence of modified E and K chromosomes, the various translocation complexes under study could have arisen by means of a single interchange for each complex. Huadquina or Coripata and their counterparts would then become the " stand-

ard" line, with Apolo Red and Ceniza each differing from it by two independent interchanges one of which involves a common chromosome (S). A single translocation would account for the ring of four by which Cerro Alegre differs from Huadquina. On this hypothesis the 6-complex of Pabur would be produced by a translocation involving an E or K chromosome and a chromosome as yet not positively identified. The more complex varieties, Chinchao and Cuba White could have arisen as double interchange products, the two rings of four being of independent origin in each case. Preliminary results indicate that Trujillo is probably closely allied to Cerro Alegre. The variety Sandia shows a minimum of chromosomal association in hybrids involving Huadquina but analyses of tester hybrids are admittedly inadequate to place this complex variety in its proper relationship.

Although the varieties Chinchao and Pabur have been subjected to analysis using all 24 of the monosomic types, one member of a chromosomal association of each remains unidentified. There is, therefore, a strong possibility that the monosomic cultures are not completely of the primary type. At first, the D chromosome was regarded as being a member of the 6-complex of Pabur but subsequent studies have indicated that in ring-forming hybrids generally, haplo-D cells are frequently characterized by a breakdown of the ring of chromosomes. In haplo-D Purpurea the P.M.C. contain a high frequency of configurations of $22_{II} + 3$. The extra chromosome in each generation is introduced from the normal inbred line yet the condition persists. If it should become possible to obtain the primary type corresponding to this presumably modified monosomic the situation outlined above may be resolved.

SUMMARY

Differences in chromosome structure among 55 varieties of *N. tabacum* have been analyzed in an attempt to obtain further information as to the genetic composition of the species. Hybrids between Purpurea, a highly inbred line, and the other 54 varieties reveal that the chromosomes of 25 large-seeded, more or less commercial types are structurally similar to those of Purpurea. Homozygous, relatively interchanged chromosomes are present in 29 varieties which are characterized by relatively small seeds and weedier growth habit.

Cytological studies of hybrids between four selected tester varieties and the above 29 give evidence of 11 different classes with regard to the configurations obtained. These involve ten different complexes of four and six chromosomes.

Monosomic analysis of the complexes has made possible the identification of all but two of the interchanged chromosomes. In nearly all cases the components of a particular association are members of the same genome.

Results of cytological studies of backcrosses indicate that transmission of certain monosomics through the pollen may be higher than expected on the basis of previous work.

Consideration is given to the problem of the structural type from which the other varieties have been derived.

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GENE LINEARITY AND NEGATIVE INTERFERENCE IN CROSSES OF ESCHERICHIA COLI

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THE discovery of sexual reproduction and genetic recombination in *Escherichia coli* strain K-12 was made possible by controlled crosses of mutant lines, differing in growth factor requirements (TATUM and LEDERBERG 1947; LEDERBERG 1947) or resistance to drugs and phages (LEDERBERG 1950). Where reversion rates are low, recovery of nutritionally independent (prototroph) or doubly resistant cells from mixedly infected selecting media is dependent upon genetic exchange between complementary mutant strains. Thus in crosses of threonine leucine dependent (diauxotrophic) W-677 (T^-L^-) and biotin methionine dependent 58-161 (B^-M^-) only prototroph recombinants ($B^+M^+T^+L^+$) are recovered as colonies from minimal medium. The segregation of additional unselected markers into the selected recombinant class may be studied.

Using these methods LEDERBERG in 1947 showed that *B*, *M*, *L*, *T*, and unselected loci controlling lactose fermentation (*Lac*) and bacteriophage T_1 resistance (V_1) are linked according to the map (*BM*) 22 *Lac* 38 V_1 20 (*LT*). The order of loci within brackets was not established. Since recombination of (*BM*) and (*LT*) is obligatory, map distances had to be based on the relative frequencies of single and triple crossovers. Interference cannot be estimated where only two loci are studied between selected loci (BAILEY 1951) and where a third one (V_6) was considered there were strong indications of interference.

Further linkage data on derivatives of LEDERBERG's lines were published by NEWCOMBE and NYHOLM (1950a, b, and c). A locus controlling streptomycin response recombined to 5 or 10 percent with (*BM*) and segregation independently of *Lac* and V_1 placed it to the left of (*BM*). Various fermentation loci (*Mal, Xyl, Gal, Ara*) apparently located between *S* and (*BM*) could not be mapped unequivocally. Assuming the existence of linearity, it appeared that multiple crossovers were in excess (negative interference). Similarly high coincidence in crossing over between fermentation loci was also observed by LEDERBERG, CAVALLI and ALLEN (LEDERBERG *et al.* 1951; CAVALLI 1950).

Since these anomalies left bacterial linearity unproven, it was decided to reinvestigate the region to the right of (*BM*) involving the loci *B*, *M*, *Lac*, V_1, *L*, and *T*. The order of *L* and *T* would be decided on the basis that monoauxotrophs for the factor mapped to the right should show the better agreement with prototroph segregation of *Lac* and V_1. Critical data would be

obtained by supplementing crosses with leucine and threonine one at a time and scoring for growth factor requirements as well as fermentation and resistance characteristics. Furthermore by supplementing both threonine and leucine and selecting for recombinants between S and (BM) one could recover all recombination classes of Lac, V_1, L, and T and test for linearity and interference in a progeny unbiased except for possible interference across (BM). The results and conclusions from unsupplemented, singly supplemented and doubly supplemented crosses are described in the following report.

MATERIAL AND METHODS

The parental lines were LEDERBERG's W-677 and a streptomycin resistant mutant (NEWCOMBE and NYHOLM) of LEDERBERG's 59-161 of *E. coli* K-12. These lines were reported to possess the following mutant characters:

W-677: Lac^-, Mal^-, Xyl^-, Gal^-, Ara^-, $V_1{}^r$, $B_1{}^-$, T^-, L^-
58-161 S^r: B^-, M^-, S^r

where the symbols represent, respectively, lactose, maltose, xylose, galactose, and arabinose non-fermentation, bacteriophage T_1 resistance, thiamin, threonine, leucine, biotin, and methionine requirements, and streptomycin resistance.

The characteristics of the parent lines were verified with the following exceptions:

1) The thiamin requirement of W-677 was not clearly indicated. Nonetheless, to safeguard against possible selective advantages of $B_1{}^+$ recombinants, a supplement of 2.5 μg/l was added to all cross and test media. Thus, henceforth, when minimal medium is spoken of, this means thiamin minimal medium.

2) The leucine requirement of W-677 could only be filled with L-leucine. This, for supplemented crosses, needs to be entirely free of methionine with which most commercial samples are contaminated. Full growth is obtained with a supplement of 18 μg/ml. D-leucine is inhibitory, and growth fails in all concentrations of the racemic mixture.

3) The biotin requirement of 58-161 could not be demonstrated. In the presence of methionine, the addition of biotin to the medium did not improve growth of 58-161 and the addition of raw egg white, containing avidin, did not inhibit growth. After considerable experimentation it was concluded that in our crosses, B is not operative as a selecting locus. Thus, in unsupplemented crosses, only the loci M, T, and L are relied upon for selection of recombinants. Fortunately the reversion rate to methionine independence is very low, and no prototrophs have been found in controls.

Unsupplemented crosses were made by spreading washed suspensions of the two parents (M^- and T^-L^-) on minimal agar (LEDERBERG 1947). After 48 hours incubation prototroph recombinants ($M^+T^+L^+$) were picked, diluted, and test-streaked directly on eosine-methylene blue (EMB) agar plates with appropriate sugars and cross-streaks of bacteriophage and streptomycin.

Threonine supplemented crosses were carried out in an analogous manner on minimal agar supplemented with 18 μg/ml of threonine. Recombinant

colonies ($M^+T^+L^+$ and $M^+T^-L^+$) were tested as in unsupplemented crosses, but in addition were scored for threonine requirement by streaking on minimal agar with and without threonine supplement.

Leucine supplemented crosses were made on minimal agar containing 18 μg/ml of leucine. Recombinants from this type of cross had to be picked against a heavy background growth which could not be eliminated. Categorizing of recombinant colonies ($M^+T^+L^+$ and $M^+T^+L^-$) was as in unsupplemented crosses. The leucine requirement was tested for by streaking on minimal agar with and without leucine.

Threonine-leucine supplemented crosses were screened for S^rM^+ recombinants. The washed parents were plated together on doubly supplemented minimal medium. Under these conditions W-677 grew very rapidly. Following eight hours preliminary incubation for initiation of zygote formation and recombination, cross plates were sprayed with 20 mg of streptomycin. After a further 36 hours incubation S^rM^+ colonies were picked from a perfectly clear background. These were subjected to single colony reisolation on the cross medium to eliminate W-677 parental contaminants. "Purified" colonies ($M^+T^+L^+$, $M^+T^-L^+$, $M^+T^+L^-$, $M^+T^-L^-$) were then tested for fermentation and resistance characteristics on the standard set of EMB plates, and for growth factor requirements on four minimal agar plates containing, respectively, no supplement, threonine alone, leucine alone, and both threonine and leucine.

RESULTS AND CONCLUSIONS

The unsupplemented cross

When W-677 and 58-161 are crossed on minimal agar, four types of progeny are recovered through recombination in the right end of the linkage map. These are due to single crossovers in regions a ($Lac^+V_1^s$), b ($Lac^-V_1^s$), c ($Lac^-V_1^r$), and triple crossing over in abc ($Lac^+V_1^r$) (see figure 1). Three

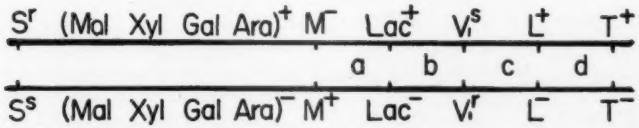

FIGURE 1.—Linear sequence of gene loci M, Lac, V_1, L, T in lines of *Escherichia coli* K-12. (Above, 58-161 S^r; below, W-677.)

replicate experiments were in close agreement and the totals only are shown in table 1.

Contamination with prototroph mutants is considered negligible since: (1) in regard to 58-161, the reversion rate to methionine independence is low and $S^r(Mal\ Xyl\ Gal\ Ara)^+$ colonies are infrequent and their proportion no higher among the $Lac^+V_1^s$ than among the other three classes combined; and (2) in regard to W-677 a simultaneous reversion to leucine and threonine independence is improbable and cannot be demonstrated when large populations of this parent alone are plated on minimal medium.

TABLE 1

Segregation of Lac, V_1, T, and L in unsupplemented, threonine supplemented, leucine supplemented and threonine leucine supplemented crosses of W-677 × 58-161 S.

Recombination classes	Supplement							
	None		T		L		TL	
	Number	Fraction	Number	Fraction	Number	Fraction	Number	Fraction
T^+L^+ $Lac^+V_1^s$	409	.337	286	.343	343	.500	40	.288
$Lac^+V_1^r$	39	.032	32	.038	20	.029	8	.058
$Lac^-V_1^s$	497	.410	310	.372	191	.278	57	.410
$Lac^-V_1^r$	267	.220	206	.247	132	.192	34	.245
Total	1212	.999	834	1.000	686	.999	139	1.001
T^-L^+ $Lac^+V_1^s$			60	.165			5	.227
$Lac^+V_1^r$			9	.025			0	.000
$Lac^-V_1^s$			138	.380			9	.409
$Lac^-V_1^r$			156	.430			8	.364
Total			363	1.000			22	1.000
T^+L^- $Lac^+V_1^s$					5	.013	7	.137
$Lac^+V_1^r$					8	.021	6	.118
$Lac^-V_1^s$					7	.018	6	.118
$Lac^-V_1^r$					366	.948	32	.627
Total					386	1.000	51	1.000
T^-L^- $Lac^+V_1^s$							14	.012
$Lac^+V_1^r$							20	.017
$Lac^-V_1^s$							39	.033
$Lac^-V_1^r$							1115	.939
Total							1188	1.001

Assuming absence of interference, recombination percentages a, b, and c were calculated from the four class totals from the equations:

1. $a - ab - ac + abc = 40900/1212\,x$
2. $b - ab - bc + abc = 49700/1212\,x$
3. $c - ac - bc + abc = 26700/1212\,x$
4. $abc = 3900/1212\,x$

where x is a correction factor for eliminated non- and double crossovers. These equations yield the values a = 25.1, b = 30.4, c = 19.0, (x = 2.22), in fair accord with LEDERBERG's mapping.

The T-supplemented cross

Since *L* and *T* are linked (LEDERBERG 1947) the percentage frequency of L^+T^- recombinants will equal the percentage recombination over *L-T*, provided there is no interference. On the same assumption, if *T* is to the right, the *Lac* V_1 segregations will be alike among the L^+T^+ and the L^+T^-. If *T* is to the left, a relative excess of $Lac^-V_1^r$, the only single crossover progeny, is expected among the L^+T^-. In five replicate experiments the proportion and genetic composition of the L^+T^- varied somewhat, presumably through

the inclusion of variable numbers of W-677 reverts to leucine independence. Since heterogeneity was not such as to affect conclusions on the sequence of *L* and *T*, the totals only are shown in table 1.

The data indicate approximately 30 percent recombination over *L*-*T*. The *Lac* V_1 segregations of the L^+T^+ agree very well with those from the unsupplemented cross but among the L^+T^- there is a significant excess of $Lac^-V_1{}^r$. This excess appears inadequate to justify mapping *T* to the left of *L* since this procedure would render one triple crossover type ($Lac^-V_1{}^s$) almost as common as the presumed single. On the other hand, mapping *T* to the right of *L*, the excess of $Lac^-V_1{}^r$ among the L^+T^- might be accounted for by (1) contamination of the cross with a considerable number of W-677 reverts to leucine independence and (2) the operation of negative interference, double recombinants over cd being more common than expected. Reversions were anticipated since controls inoculated only with W-677 yield colonies. That they did occur is indicated by the relative excess of S^s(*Mal Xyl Gal Ara*)$^-$ among the $Lac^-V_1{}^rL^+T^-$. Reality of the interference phenomenon is supported by the relative excess of $Lac^-V_1{}^s$ (bd) over $Lac^+V_1{}^s$ (ad) among the threonine dependents. Thus, since there is experimental support for the efficacy of both postulated mechanism, *T* is provisionally mapped to the right of *L*.

The effect of mutants on the estimate of map distance d may be eliminated by ignoring the $Lac^-V_1{}^r$ both among the L^+T^+ and the L^+T^-. This procedure yields 207 T^- colonies in a total progeny of 835 and the recombination percentage d = 25, ignoring interference.

The L-supplemented cross

If the sequence of *L* and *T* inferred above is correct the *L*-cross is expected to yield an abundance of $Lac^-V_1{}^r$ among the leucine dependents. Five replicate experiments differed somewhat in the proportion of L^+T^+ and L^+T^- and the *Lac* V_1 segregations within both groups. Variations were not such as to affect seriously mapping of *L* and *T* and totals only are shown in table 1.

The prototrophs may be considered first. Their *Lac* V_1 segregations should be independent of the crossing medium, the excess of $Lac^+V_1{}^s$ on leucine minimal medium (table 1) therefore requires an explanation. It is not due to excessive methionine reversion in the presence of leucine as there is no change in the proportion of $Lac^+V_1{}^s$ that are (*Mal Xyl Gal Ara*)$^+$. It would appear, therefore, that either certain L^+T^+ recombinants (largely $Lac^+V_1{}^s$) which form colonies in the presence of leucine do not do so in its absence or, more probably, that some L^+T^+ (largely other than $Lac^+V_1{}^s$) which grow on unsupplemented medium are inhibited by leucine. On the likeliest interpretation the prototrophs comprise two genetic types differing in a modifier allele (L_m) such that $L^+L_m{}^-T^+$ is inhibited by leucine while $L^+L_m{}^+T^+$ is not. The original K-12 (and the derived 58-161) grow both in the presence and absence of leucine ($L^+L_m{}^+$). Irradiation presumably produced an $L^-L_m{}^+$ stock which did not grow in the absence of leucine and which grew poorly in its

presence but sufficiently so to collect the L_m^- modifier mutation which enabled it to grow well. This is the condition of W-677 ($L^-L_m^-$). Interaction of L and L_m is such that the crossover product $L^+L_m^-$ grows only in the absence of leucine. The elimination in the L plates of approximately one half the $Lac^-V_1^s$ recombinants suggests that the locus L_m is halfway between *Lac* and V_1. This, however, is not conclusive evidence of its position. The basic interpretation in terms of a modifier locus is borne out by the results of the doubly supplemented cross where it is found that certain prototrophs grow well on minimal medium, grow moderately well on TL-supplemented medium, but do not grow at all on L-supplemented medium. Among these types $Lac^-V_1^s$ and $Lac^-V_1^r$ predominate.

Turning next to the L^-T^+, the expected vast excess of $Lac^-V_1^r$ is indeed observed; in fact, in one of five replicate experiments no other types were obtained. This finding immediately supports mapping L to the left of T. However, two phenomena must be taken into account which contribute $Lac^-V_1^r$ by means other than the linkage *M, Lac,* V_1, *L, T*. These are: (1) segregation of the modifier L_m favoring the $Lac^-V_1^r$ with the parental combination $L^-L_m^-$; and (2) reversion of W-677 to threonine independence. The latter effect is significant as judged by (a) the number of $Lac^-V_1^r$ that are S^s(*Mal Xyl Gal Ara*)$^-$ and (b) crude mutation rate studies which indicate approximately 10^{-8} reversions per bacterial division. That appreciable numbers of $Lac^-V_1^r$ are not mutants is clear since 5 percent carry one or more of the 58-161 markers in addition to T^+. Furthermore, with either sequence of L and T, the proportion of L^+T^- in the T-cross (30 percent) and of L^-T^+ in the L-cross (36 percent) should be of the same order of magnitude (ad + bd + cd + abcd against d + abd + acd + bcd). This relation cannot be maintained if almost all $Lac^-V_1^r$ are considered mutants.

It is concluded, therefore, that the L-cross yields a real excess of $Lac^-V_1^r$ consistent with the mapping of L to the left of T. This conclusion is borne out by the results of the TL-cross described in the next section.

The TL-supplemented cross

In the doubly supplemented cross S^rM^+ recombinants are selected and the confusing effects of reversions to threonine and leucine independence are eliminated. On the other hand W-677 S^r mutants are scored as non-crossovers and 58-161 methionine reverts as single recombinants in a. The crossover distribution in the freely segregating region to the right of M is recorded for five replicate experiments in table 2 on the provisional sequence of figure 1. Possible mutant types were scored as non-c.o./$S^s - S^r$ ($Sugar^-V_1^rL^-T^-$) and single c.o. a/$M^- - M^+$ ($Sugar^+V_1^sL^+T^+$) as opposed to *bona fide* non-c.o. and single c.o. a with mixed parental fermentation markers.

An attempt was made to determine the proportion of mutants and segregants in the two possible mutant classes. The number of recombinants among the 25 single c.o. a/$M^- - M^+$ was estimated using the following ratios of

TABLE 2

Segregation of Lac, V_L, L, and T in S^rM^+ recombinants from threonine leucine supplemented cross. (5 replicate experiments. For crossover regions a, b, c, d, see figure 1.)

Crossover (CO) type	Experiment no.					Total	Total minus mutants
	1	2	3	4	5		
0 CO/S^s-S^r	80	112	37	152	67	448	
0 CO	248	117	69	162	79	675	1115
0 CO (all)							1115
1 CO a/M^--M^+	4	0	14	2	5	25	
1 CO a	8	9	3	4	4	28	40
1 CO b	6	17	8	20	6	57	57
1 CO c	3	10	7	5	9	34	34
1 CO d	11	5	3	7	6	32	32
1 CO (all)							163
2 CO ab	5	2	1	7	5	20	20
2 CO ac	5	1	0	2	6	14	14
2 CO ad	2	0	0	2	1	5	5
2 CO bc	7	6	4	15	7	39	39
2 CO bd	4	1	1	3	0	9	9
2 CO cd	4	3	0	0	1	8	8
2 CO (all)							95
3 CO abc	0	2	3	3	0	8	8
3 CO abd	1	3	0	1	1	6	6
3 CO acd	1	3	0	2	1	7	7
3 CO bcd	0	2	0	2	2	6	6
3 CO (all)							27
4 CO (all)	0	0	0	0	0	0	0
Total	389	293	150	389	200	1421	1400

(*Mal Xyl Gal Ara*)$^+$/total in known recombination classes: single c.o. a (incl. mutants): 25/53, single c.o. b: 12/57, single c.o. c: 4/34, single c.o. d: 3/32, all doubles: 20/95, all triples 2/27. On the basis of the single c.o. b at least 12 of the "possible mutants" are regarded as recombinants (raising the total single c.o. a to 40) and the remaining 13 as mutants. Most of the latter occurred in the single run No. 3 in which, for unknown reasons, the ratio of mutants and recombinants appears shifted.

A direct attempt was made to estimate the relative frequencies of S^r mutants and recombinants. In experiment No. 4, 20 TL plates were inoculated with both parents, another six plates were inoculated with W-677 ($S^s M^+$) only. On spraying with streptomycin the cross plates yielded 500 colonies. The controls yielded no S^r mutants. Similarly, in experiment No. 5, 10 cross plates gave 200 colonies and eight control plates gave two independent S^r mutants.

Thus, whereas a total of 30 cross plates yielded 700 well separated colonies, the 14 control plates gave only two mutants and an experiment of the order of the total of table 2 may be expected to include only about eight S^r mutants.

An indirect approach also was made using the following ratios of (*Gal Ara Xyl Mal*)$^-$/total in known recombinant groups: non c.o. (incl. mutants): 448/1123 = .39, all singles (excl. a): 27/123 = .22, all doubles: 26/95 = .27, all triples: 8/27 = .30. The ratio does not differ significantly among the single, double, and triple recombinants and averages .25. If this is valid also for the noncrossover segregants, only 900 of the non-c.o./$S^s - S^r$ are recombinants and 223 must be eliminated as mutants. However, if there is negative interference and particularly an excess of non-crossovers through irregular and incomplete pairing, the number of mutants would be overestimated by this procedure. More weight is attached, therefore, to the direct evidence of the control experiment and in table 2 only eight of the 448 non-c.o./$S^s - S^r$ are eliminated as mutants. The implied very low mutation rate is in accord with the experience of NEWCOMBE and HAWIRKO (1949) who, with maximum population densities on complete media, had difficulty getting S^r mutants.

The results of the doubly supplemented cross as shown in the last column of table 2 support the linear sequence of *M Lac* V_1 *L T* inferred from the singly supplemented crosses, for: (1) of the 16 possible combinations of *Lac* V_1 *L* and *T*, the only one not found in 1400 segregants is $Lac^+V_1{}^rL^+T^-$, the presumed quadruple crossover, all others being found at least five times; and (2) here, where reversions to threonine and leucine independence are not selected for, there is a distinct excess of $Lac^-V_1{}^r$ among the L^-T^+ (table 1), whereas the *Lac* V_1 segregations of the L^+T^- are like those of the prototrophs. However, while the linear sequence *M Lac* V_1 *L T* is confirmed, the provisional assumption of absence of interferences can be shown to be inadequate and map distances must be revised. These points are considered in the following discussion.

DISCUSSION

In the TL-supplemented cross S^rM^+ recombinants alone survive and all recombination classes involving *Lac*, V_1, *L*, and *T* (non-, single, double, triple, quadruple crossovers) are recovered. Valid estimates of crossover frequencies are obtained only if there is no interference across *M*. The best prospect of minimizing and estimating the possible effects of limited interference lies in the study of recombination distal to selected loci.

Using this approach NEWCOMBE and NYHOLM (1950c) attempted to place the loci *S Mal Xyl Gal Ara* in a linear array to the left of *M*. However, depending upon the method (1, interaction with *Lac;* 2, recombination with *S;* 3, recombination with *M;* 4, comparison of factors three at a time, and choosing as middle factor that one undergoing recombination least often with respect to the other two), five different and conflicting sequences were obtained (two from method 4). The number of colonies in their "unselected" crosses was too small to base a sequence on the identification of the quintuple

crossover type, but the finding of all *Gal Ara Xyl Mal* combinations among the S^r is contrary to the suggested sequence *S* (*Mal Gal Ara Xyl*) *M* and the demonstrated interaction across *M* of all fermentation loci, and *Lac* is in itself anomalous. Since no sequence could be chosen as being the most probable, the authors proceeded to show that, granting linearity, their evidence for conflicting sequence implied an excess of multiple crossovers, no matter which sequence is, in fact, the correct one.

In the present study the sequence *M Lac* V_1 *L T* is strongly indicated, yet there is evidence of the same kind of anomaly which was observed by NEWCOMBE and NYHOLM. (1) Among the 1400 recombinants of table 2 recombination between *M* and the unselected loci *Lac*, V_1, *L*, and *T* occurred with the following frequencies: *M-Lac:* 100, *M-*V_1*:* 177, *M-L:* 161, *M-T:* 190. Thus frequency of recombination with *M* yields the sequence *M Lac L* V_1 *T*, contrary to the linked segregations of *Lac* and V_1 in unsupplemented crosses. (2) Determining the sequence by the "middle locus method" nine of the ten possible comparisons of loci three at a time are compatible with the sequence *M Lac* V_1 *L T*, but the tenth comparison places *L* between *M* and V_1. In both cases conflict with the accepted sequence is largely due to bc being more common than expected. Since excessive numbers of bc were introduced through the single run No. 4 no assurance is felt that the evidence for conflicting sequence is significant.

On the other hand, striking interference effects are revealed in the relative frequencies of non-, single, double and triple recombinants. In 1400 recombinants 100 crossovers were scored in a, 145 in b, 116 in c, and 73 in d. Thus, the TL-cross yields the recombination percentages a = 7.1, b = 10.4, c = 8.3, d = 5.2, as compared to a = 25.1, b = 30.4, c = 19.0, d = 25.0 from non- and T-supplemented crosses assuming absence of interference and differential death. In table 3 the expected and observed frequencies of all recombination classes are compared. It is clear that there is (1) a relative deficit of all single recombinants, and (2) a tendency for multiple crossovers to occur close together, that is in terms of survivors crossing over is rare, but where one crossover occurs it tends to be accompanied by a second and a third nearby.

NEWCOMBE and NYHOLM have attempted to decide whether excess survival or excess formation of multiple recombinants is responsible for this type of anomalous distribution. Among genetic mechanisms leading to selective survival they have discussed: ring chromosomes, position of loci between lethals or in inversions, and loss phenomena from zygotes carrying the *Het*-factor (LEDERBERG 1949, and 1950b). They have concluded that no simple structural or genic modification of chromosomes would alone account for the anomalous segregations to the left of *M*.

However, more unorthodox complexities may be involved. Thus LEDERBERG *et al.* (1951) propose that zygotes of W-677 and 58-161 are interchange hybrids, a cross-shaped quadrivalent pairing configuration being the physical counterpart of a branched four-armed linkage map. This suggestion gains

TABLE 3

Segregations compared with expectations: 1. in absence of interference 2. with excess of non-crossovers (1000/1400) due to non-pairing. (For crossover regions a, b, c, d, see figure 1.)

Crossover type	Observed	Expected			
		Non-interference		Excess non-CO	
	Number	Number	χ^2	Number	χ^2
O (all)	1115	1013.07	10.26	1111.00	.014
a	40	77.93	18.46	37.00	.243
b	57	117.05	30.81	63.12	.593
c	34	91.53	36.16	45.36	2.845
d	32	55.73	10.10	24.76	2.117
all single	163	342.24		170.24	
ab	20	9.00	13.44	21.04	.051
ac	14	7.04	6.88	15.12	.083
ad	5	4.29	.12	8.28	1.300
bc	39	10.57	76.47	25.76	6.805
bd	9	6.44	1.02	14.12	1.856
cd	8	5.03	1.75	10.12	.444
all double	95	42.37		94.44	
abc	8	.81	63.83	8.60	.042
abd	6	.50	60.50	4.68	.372
acd	7	.39	112.03	3.36	3.943
bcd	6	.58	50.66	5.76	.010
all triple	27	2.28		22.40	
abcd (all quadruple)	0	.04	.04	1.92	1.922
Total	1400	1400.00	492.53[1]	1400.00	22.640[1]

[1] 15 degrees of freedom.

substance from DELAMATER's evidence (1951) that *E. coli* K-12 has two chromosomes per nucleus. Interchange heterozygosity might explain conflicting indications of sequence and the nearly equal non-linear linkage of several loci with *M* (one breakage point near *M*). It does not account for the interaction between *Lac* and other fermentation loci nor for the significant interaction between all of the loci studied by NEWCOMBE and NYHOLM. In the present study it is possible to place the loci *M*, *Lac*, V_1, *L*, and *T* in an unambiguous sequence, and interaction among these loci is of a higher order than between them and other loci. Thus, these factors would be linearly arrayed on one arm of the linkage map, and interchange hybridity is not relevant to an analysis of their recombination.

A second complexity which may contribute to linkage anomalies is the as yet uninterpreted behavior of persistent heterozygotes. The relevant published facts are as follows: In EMS (synthetic medium) crosses of certain stocks

(W-466, etc.) carrying the hypothetical *Het*-factor considerable numbers of persistent diploids are discovered (10 percent). By segregation and reversion tests on EMB each diploid clone is seen to be heterozygous for certain factors, homozygous for others and hemizygous for yet others. Thus, genetic recombination and elimination precede diploidization. Since clonal segregants are of more than two or even four types, recombination must again occur in the formation of haplonts. Furthermore, segregations from the heterozygous state deviate widely from the 1:1 ratio. Diverse diploid clones isolated from the same cross, differ in the distribution of, at least, homo- and heterozygosity, and in the segregation of identical heterozygous markers. Segregants backcrossed to parental standard stocks show altered linkage relations. Special screening techniques have allowed the identification in standard crosses (W-677 and 58-161) of rare (.1 percent of prototrophs) diploids, qualitatively like those of the *Het*-series.

On the relevance of these aberrant heterozygotes one may take one of two stands. (1) It is possible that a similar duplex meiosis and gene loss precede the segregation of transient zygotes in standard crosses. This might be inferred from the abnormal linkage relations in backcrosses of 58-161 and L^-T^- recombinants (ostensibly non-*Hets*) extracted from TL-crosses (LEDERBERG, personal communication). Similarly it is suggestive that selection in appropriately supplemented crosses of complementary diauxotrophic recombinants yields complementary segregations for some loci (*Lac*, V_1) but not for others (*Mal*) which are generally eliminated in the persistent diploids (data of P. FRIED, cited in LEDERBERG 1951). If standard and *Het* segregants differ only in the duration of the diplophase and if there is in addition structural hybridity, attack on the linkage problem must be renewed with unrelated stocks, and the present discussion is premature. (2) It is possible, however, that the nuclear cycles of *Het* and standard zygotes are entirely different, the former being initiated only by special crossover configurations in structurally hybrid zygotes. Thus crossing over in relatively inverted segments might lead to bridge formation and delay of cytoplasmic division resulting, on bridge breakage, in two complementary hypodiploid cells. One or both of these might propagate mitotically in diplophase and eventually segregate haplonts meiotically. Such diploids would be homo-, hemi- or heterozygous for any given locus depending upon crossover and bridge breakage relations. Alternatively, if the standard zygote is an interchange hybrid, the *Het*-cycle might be started simply by a partial (3:1) nondisjunction. However, since elimination affects only certain loci (*Mal*, *S*) and since the *Het*-character originated in a single mutation-like step, non-recurrent in the parent strain, nondisjunction is likely to be directed by a second superimposed chromosomal change such as an inversion. It should be noted that with a system of double hybridity the *Het*-character might be perpetuated in inbred clones and haploid progeny could exhibit altered linkage relations whether they had persisted for a time as heterozygotes or not.

If this general interpretation is correct, then where persistent diploids are rare (W-677 × 59-161) linkage anomalies are quite independent of chromosomal aberrations. This is the view adopted by us at the present. This discussion will be continued, therefore, on the contention that the excess of multiple crossovers between all loci so far studied is not due to the elimination of certain crossover types nor to life cycle complexities of the *Het*-type but reflects largely interference at the level of crossing over.

Whether there is significant interference between two regions may be judged by the calculation of interaction, *i.e.*, the product of non- and double crossovers divided by the product of single crossovers. Interactions of unity indicate absence of interference, interactions significantly smaller than unity indicate positive interference, and interactions larger than unity indicate negative interference. The six possible comparisons, taking crossover a, b, c, d, two at a time, are shown in table 4. Interactions are very much larger than

TABLE 4

Interference between crossover regions a, b, c, d two at a time. (For crossover regions a, b, c, d see figure 1.)

Crossover type	Number of segregants in paired regions					
	ab	ac	ad	bc	bd	cd
0 CO	1189	1213	1245	1192	1203	1232
1 CO (region 1)	66	71	82	92	124	95
1 CO (region 2)	111	87	55	63	52	52
2 CO	34	29	18	53	21	21
Interaction[1]	5.518	5.695	4.969	10.900	3.918	5.237
Chi squared[2]	68.76	64.37	38.21	170.34	28.57	43.09

[1]Interaction equals the product of the non- and double crossovers divided by the product of the single crossovers. Where the interaction is significantly greater than 1 there is negative interference.

[2]From 2 × 2 tables. $\chi^2 = 6.6$ is significant at the 1 per cent level.

unity in every case. Thus, the observed crossover distributions in table 3 are formally correctly described as showing negative interference.

Two biological models are visualized which individually or jointly would give negative interference. (1) It may be assumed that crossing over in paired regions is free of interference but pairing itself is irregular and incomplete through random initiation and the operation of a time limit, or through structural hybridity following mutagenic treatment. Thus zygotes may undergo pairing both in *S-M* and *M-T*, or in *S-M* or *M-T* alone, or in neither. Unselected progeny, if recoverable, would show a degree of interference, negative for double crossovers in adjacent regions and positive for doubles far apart. Selection of $M^+T^+L^+$ (prototroph recovery technique) would yield an overestimate of map distances through exclusion of zygotes not paired in *M-T*. If pairing were all or none in *M-T*, there would be no internal evidence of interference but if it occurred in smaller variable blocks prototroph progeny would

show negative interference but no actual numerical excess of doubles over either single. On the other hand, in S^rM^+ selections, zygotes with pairing restricted to *S-M* will produce non-crossovers only and will largely be responsible for the apparent negative interference. Regardless of the frequency of extra non-crossovers from this source double crossovers will not exceed singles. Crossover frequencies throughout *M-T* may be underestimated and will certainly appear lower than when recombination is made obligatory over the region in which crossing over is scored (unsupplemented cross). (2) Alternatively it may be assumed that pairing is complete but that the occurrence of one crossover directly increases the probability of another occurring nearby through weakening of the chromosome strand, production of extra tension, or other means. Thus in relation to single crossovers and segmental crossover frequencies there will be too many non- and double crossovers. If crossing over in bacteria were four-strand, the phenomenon known as negative chromatid interference in higher organisms would provide a model. Depending upon the magnitude of the effect, double crossovers may or may not exceed one or both single types.

It is difficult to establish criteria which discriminate between these interpretations. Excess multiple crossing over can only be proved where there is an excess of double crossovers over at least one of the two single types. The corresponding tests are negative for the present data no matter which regions are paired (table 4).

Conversely in the TL-cross the adequacy of the incomplete pairing hypothesis may be tested by ignoring the non-crossovers and considering the interrelations of singles, doubles and triples. If there is no interference, and pairing in *M-T* is all or none, for each region the product of singles and triples divided by the product of the doubles should be unity. Tests were carried out for regions a, b, c, and d, three at a time and compounded in all possible ways. The interactions are generally close to unity and where they deviate they are sometimes larger and sometimes smaller, the mean being .94. In the last columns of table 3, the specific hypothesis is tested that pairing had occurred in 400/1400 zygotes both in *S-M* and *M-T*, and in the remaining 1000 zygotes in *S-M* only. The agreement between the observed and expected distribution is surprisingly good.

Main support for excess multiple crossovers comes at present from the data of NEWCOMBE and NYHOLM. It is possible that conflicts of sequence may be resolved by accommodation of loci in a branched interchange map (LEDERBERG *et al.* 1951). In such a system unselected loci in any two arms should be linearly linked, whatever the position of selected loci. Thus, mapping (from the free ends) *T L* V_1 *Lac M* on one arm, *Mtl* (Mannitol), *Xyl* on a second, and *S*, *Mal* on a third, the following three assemblies should meet tests of linearity: (1) *T L* V_1 *Lac M; Mal S.* (2) *T L* V_1 *Lac M; Xyl Mtl.* (3) *S Mal; Xyl Mtl.* With markers in all four arms, six linear and mutually reconcilable linkage assemblages are expected. If such mapping should prove to eliminate

ambiguities of sequence it would still leave evidence of residual interference quite similar to that obtained in the present work. To give just one example; where *S-Mal-Xyl* segregated in "unselected crosses" NEWCOMBE and NYHOLM found 2224 non-crossovers, 62 single crossovers of one type and 37 of the other, and 38 double crossovers. Thus, if allowance is made for the possibility that in the study of NEWCOMBE and NYHOLM markers were distributed in several arms of a branched map while in the present study, they were confined to one, anomalies in segregation might become comparable and explicable by irregular pairing alone. On the other hand excess multiple crossing over alone or in conjunction with irregular pairing is not excluded in either study. In view of these uncertainties map distances for unselected crosses cannot be inferred from the present data. If incomplete pairing is involved prototroph recovery yields overestimates and SM selection may yield underestimates. If excess multiple crossing over is responsible even SM selections may give overestimates according to the degree of negative interference across *M*. In either case uncorrected mapping from unsupplemented crosses leads to exaggerated map distances. Thus LEDERBERG's 1947 map is not valid for unselected progeny. On the other hand, the existence of linearity which had to be assumed by NEWCOMBE and NYHOLM appears consolidated for the loci *M-Lac-*V_1*-L-T* through the relative insignificance of anomalies in segregation.

SUMMARY

Segregation of the linked gene loci *M*, *Lac*, V_1, *L*, and *T* was studied in crosses of *E. coli* strain K-12. Evidence from all crosses unambiguously supports the above linear seriation. In a critical five point cross recombination over the region *S-M*, not overlapping *M-T*, was made obligatory and all recombination classes involving *M*, *Lac*, V_1, *L*, and *T* could be recovered. The distribution of recombinants was anomalous in that, relative to segmental crossover frequencies, there was an excess of multiple crossovers. This could not be accounted for by the familiar consequences of crossing over in a structurally changed chromosome segment. The view is favored that the apparent excess of multiple crossovers is due to an excess of non-crossovers through irregular and incomplete pairing, although negative interference in completely paired chromosome regions is not ruled out.

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THE THEORETICAL VARIANCE WITHIN AND AMONG SUBDIVISIONS OF A POPULATION THAT IS IN A STEADY STATE*[1]

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IN a population that is subdivided into more or less isolated strains, the total genetic variance (σ_T^2) of a character that depends on multiple alleles at multiple loci with additive effects within and among the loci, can easily be analyzed into the variance of strain means (σ_m^2) and the average variance within strains (σ_w^2) in terms of the inbreeding coefficient F, and the variance, $\sigma_0^2 = 2\bar{q}(1-\bar{q})$, expected under panmixia with the same mean gene frequency, $\bar{q}$ (WRIGHT 1951).

$$\sigma_w^2 = (1 - F)\,\sigma_0^2 \tag{1}$$

$$\sigma_m^2 = 2F\,\sigma_0^2 \tag{2}$$

$$\sigma_T^2 = (1 + F)\,\sigma_0^2 \tag{3}$$

These simple relations hold whether the strains are completely isolated and drifting toward fixation in the absence of mutation or selection, or whether a steady state has been reached in which the tendency toward fixation is balanced by a certain amount of cross breeding, mutation or selection (acting alike on both sexes).

It should be noted that if the coefficient F is used for the purpose for which it was originally introduced, the description of population structure, it cannot take cognizance of rates of mutation or selection since these are specific for each locus. In this sense, F is related to heterozygosis, variability, correlation between relatives, etc., in only those respects in which the effects of recurrent mutation and selection are negligible. It is also desirable, however, to use F statistics that relate to specific loci and these must, of course, take account of the effects of all factors. The sort of use should be clear from the context. In either case, F can be defined as the proportional approach toward homozygosis from the situation under panmixia at the same gene frequency. The present discussion will be restricted to coefficients pertaining to disomic loci, and as random mating will always be assumed within strains, the only F coefficients considered are those of individuals relative to the total population.

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If there is dominance in any degree, it is still possible to express the total variance in terms of a function of F and gene frequency, or statistics derived from these. Letting m_T and σ_T^2 be the grand average and variance of the total population, m_0 and σ_0^2 those of a panmictic population with the same allelic frequencies and m_1 and σ_1^2 those of a random array of completely fixed strains (WRIGHT 1951)

$$m_T = (1-F)\, m_0 + F\, m_1 \tag{4}$$

$$\sigma_T^2 = (1-F)\, \sigma_0^2 + F\, \sigma_1^2 + F(1-F)\, (m_0 - m_1)^2 \tag{5}$$

The analysis of σ_T^2 into inter- and intra-strain components cannot, however, be made in these terms, if there is any degree of dominance. There are diverse possibilities with the same gene frequency and the same value of F, depending on the nature of the distribution of gene frequencies among the strains.

ALAN ROBERTSON (1952) has recently carried through the analysis in the important case of completely isolated strains of given size, tending toward fixation without interference from mutation or selection. The distribution of gene frequencies here takes a succession of forms depending wholly on the successive inbreeding coefficients, the theoretical values of which can easily be determined. The mathematical formulae for the distribution of gene frequencies are not indeed known, but as ROBERTSON shows, it is only necessary to find the law of change of the first four moments. This he has done by means of matrix algebra.

ROBERTSON shows that there is an almost qualitative difference from the results where dominance is lacking. Thus there is a considerable *reduction* in the total variance as inbreeding increases in case the gene frequency of the recessive allele is sufficiently high and a very considerable *increase* in the variance within strains with increasing F, up to a certain point, in the case in which the gene frequency of the recessive is sufficiently low.

SUBDIVIDED POPULATIONS IN A STEADY STATE

It may be of interest to make a comparison with the results from a very different situation, that in which a steady state has been reached in a population divided into partially isolated strains, the tendency toward fixation being balanced by occasional cross breeding. This case is of primary importance in the theory that evolution consists ordinarily or second order shifts in such states of balance. The analysis is simpler since the form of the distribution of gene frequencies is known in this case.

Gene frequency is represented by q with distribution, $\phi(q)$ among strains, characterized by mean $\bar{q}$, variance σ_q^2 and higher moments. The strains are assumed to be alike in effective size (N) and other conditions. The distribution $\phi(q)$ is properly discontinuous, with values at steps of $1/(2N)$ in q. The integrals below may be considered as Stieltjes integrals applicable to step functions as well as continuous ones. The results are theoretically exact if $\phi(q)$ is an exact discontinuous distribution but are merely close approximations if continuous. Practically, of course, the irregularities in size and in other

respects in actual cases make both merely approximate models. The following four equations are merely definitions, that for F being here based on proportional approach to homozygosis.

$$\int_0^1 \phi(q)dq = 1 \tag{6}$$

$$\int_0^1 q\phi(q)dq = \bar{q} \tag{7}$$

$$\int_0^1 (q-\bar{q})^2\phi(q)dq = \sigma_q^2 \tag{8}$$

$$2\int_0^1 q(1-q)\phi(q)dq = 2\bar{q}(1-\bar{q})(1-F) \tag{9}$$

From these

$$\int_0^1 q^2\phi(q)dq = \bar{q}^2 + \sigma_q^2 = \bar{q}^2 + \bar{q}(1-\bar{q})F \tag{10}$$

The mode of analysis may be illustrated most simply by the case of no dominance, with character values 0, a and $2a$ assigned to genotypes aa, Aa and AA respectively. For a strain with zygotic array $[(1-q)a+qA]^2$, the character mean, m, is $2qa$ and the variance of the character, σ_w^2, is $2q(1-q)a^2$. For the total population, we are led at once to the results already referred to (in application here, however, merely to a pair of alleles). It is sometimes convenient to use p for $(1-q)$ for brevity.

$$\bar{m} = \int_0^1 m\phi(q)dq = 2a\int_0^1 q\phi(q)dq = 2\bar{q}a \tag{11}$$

$$\overline{\sigma_w^2} = \int_0^1 \sigma_w^2\phi(q)dq = 2a^2\int_0^1 q(1-q)\phi(q)dq = 2\bar{p}\bar{q}(1-F)a^2 \tag{12}$$

$$\sigma_m^2 = \int_0^1 (m-\bar{m})^2\phi(q)dq = 4a^2\int_0^1 q^2\phi(q)dq - \bar{m}^2 = 4\bar{p}\bar{q}Fa^2 \tag{13}$$

$$\sigma_T^2 = \overline{\sigma_w^2} + \sigma_m^2 = 2\bar{p}\bar{q}(1+F)a^2 \tag{14}$$

Consider now the case of complete dominance, letting q be the frequency of the recessive allele in a strain and a the differential effect of aa. For the mean and variance in the phenotypic array $[(1-q^2)A-+q^2aa]$ in a strain we have $m = q^2a$, $\sigma_w^2 = q^2(1-q^2)a^2$. In the total population

$$\bar{m} = a\int_0^1 q^2\phi(q)dq = (\bar{q}^2 + \bar{p}\bar{q}F)a \tag{15}$$

$$\overline{\sigma_w^2} = a^2\left[\int_0^1 q^2\phi(q)dq - \int_0^1 q^4\phi(q)dq\right] \tag{16}$$

$$\sigma_m^2 = a^2\int_0^1 q^4\phi(q)dq - \bar{m}^2 \tag{17}$$

$$\sigma_T^2 = a^2\int_0^1 q^2\phi(q)dq - \bar{m}^2 = \left[\bar{q}^2 + \bar{p}\bar{q}F\right]\left[1 - (\bar{q}^2 + \bar{p}\bar{q}F)\right]a^2 \tag{18}$$

Thus the total variance can be evaluated in terms of $\bar{q}$, F and a irrespective of the form of $\phi(q)$. The value agrees with that obtained by substituting $m_0 = \bar{q}^2a$, $\sigma_0^2 = \bar{q}^2(1-\bar{q}^2)a^2$, $m_1 = \bar{q}a$ and $\sigma_1^2 = \bar{q}(1-\bar{q})a^2$ in the more general formula cited earlier. In this case it reduces to $\bar{m}(a-\bar{m})$, but this is not true in general.

Apportionment of this total variance into $\overline{\sigma_w^2}$ and σ_m^2 requires evaluation of $\int_0^1 q^4\phi(q)dq$. ROBERTSON, as noted, found the law of change of this quantity under the cumulative effect of accidents of sampling among strains of a given

size. To deal with a population in a steady state it is necessary to find the formula of $\phi(q)$ under the postulated conditions.

If a gene frequency is subject to systematic change at the rate Δq per generation, and to random fluctuations (δq) including the effects of inbreeding, the resultant probability distribution has the formula

$$\phi(q) = (C/\sigma_{\delta_q}^2)e^{2\int(\Delta q/\sigma_{\delta_q}^2)dq}, \qquad \int_0^1 \phi(q)dq = 1 \tag{19}$$

This can be derived as solution of the FOCKER-PLANCK equation of physics for the case of a steady state (cf. KOLMOGOROFF 1935; WRIGHT 1945; MALÉCOT 1948). The present author (1938) derived it independently from the condition that mean and variance remain unchanged. This is not a complete proof but, as noted several times since, this mode of demonstration can be extended to cover the conditions that *all* moments remain unchanged. This form of the demonstration is given in the appendix.

Letting m represent the effective amount of replacement of each strain by immigrants representative of the total, the systematic tendency toward change of gene frequency is $\Delta q = -m(q - \bar{q})$. The sampling variance of 2N gametes in the array $[(1-q)A + qa]^{2N}$ is $\sigma_{\delta q}^2 = q(1-q)/2N$. Substitution in the general equation for $\phi(q)$ leads to a Beta distribution, a formula originally derived for this case by still another method (WRIGHT 1931).

$$\phi(q) = \frac{\Gamma(a)}{\Gamma(a\bar{q})\,\Gamma[a(1-\bar{q})]}\, q^{a\bar{q}-1}(1-q)^{a(1-\bar{q})-1}, \qquad a = 4Nm \tag{20}$$

The moments about zero can be easily evaluated by use of the formulae

$$\int_0^1 x^{m-1}(1-x)^{n-1}dx = \frac{\Gamma(m)\Gamma(n)}{\Gamma(m+n)}, \text{ and } \Gamma(x+1) = x\Gamma(x)$$

$$\int_0^1 \phi(q)dq = 1 \tag{21}$$

$$\mu_1' = \int_0^1 q\phi(q) = \bar{q} \tag{22}$$

$$\mu_2' = \int_0^1 q^2\phi(q) = \frac{\bar{q}(a\bar{q}+1)}{a+1} \quad (= \bar{q}^2 + \bar{p}\bar{q}F) \text{ by (10)} \tag{23}$$

$$\text{Thus } a = (1-F)/F \tag{24}$$

$$F = 1/(a+1) = 1/(4Nm+1) \tag{25}$$

$$\mu_3' = \int_0^1 q^3\phi(q)dq = \frac{\bar{q}(a\bar{q}+1)(a\bar{q}+2)}{(a+1)(a+2)} = (\bar{q}^2 + \bar{p}\bar{q}F)\left(\bar{q} + \frac{2F\bar{p}}{1+F}\right) \tag{26}$$

(27)
$$\mu_4' = \int_0^1 q^4 \phi(q)\,dq = \frac{\bar{q}(a\bar{q}+1)(a\bar{q}+2)(a\bar{q}+3)}{(a+1)(a+2)(a+3)}$$
$$= (\bar{q}^2 + \bar{p}\bar{q}F)\left(\bar{q} + \frac{2F\bar{p}}{1+F}\right)\left(\bar{q} + \frac{3F\bar{p}}{1+2F}\right)$$

From (16), (17), (23), and (27)

(28) $$\overline{\sigma_w^2} = (\bar{q}^2 + \bar{p}\bar{q}F)\left[1 - \left(\bar{q} + \frac{2F\bar{p}}{1+F}\right)\left(\bar{q} + \frac{3F\bar{p}}{1+2F}\right)\right]\alpha^2$$

(29) $$\sigma_m^2 = (\bar{q}^2 + \bar{p}\bar{q}F)\left[\left(\bar{q} + \frac{2F\bar{p}}{1+F}\right)\left(\bar{q} + \frac{3F\bar{p}}{1+2F}\right) - (\bar{q}^2 + \bar{p}\bar{q}F)\right]\alpha^2$$

(30) $$\sigma_T^2 = (\bar{q}^2 + \bar{p}\bar{q}F)[1 - (\bar{q}^2 + \bar{p}\bar{q}F)]\alpha^2 \quad (=(18))$$

Table 1 shows values of σ_T^2 for various values of $\bar{q}$ and F letting $\alpha = 1$. These apply to the case treated by ROBERTSON as well as to the steady state treated here, or to any other case of complete dominance of one of a pair of alleles. Table 2 shows the corresponding value of $\overline{\sigma_w^2}$, applicable only to a steady state in which the inbreeding effect is balanced by a linear systematic process (and dominance of one of the pair of alleles is complete). Table 3 shows the ratio of intra-strain variance under unimpeded inbreeding, as calculated by ROBERTSON's formula, to the values in table 2. ROBERTSON's formula is as follows in the terminology of this paper.

(31) $$\overline{\sigma_w^2} = \bar{p}\bar{q}\left[\left(\frac{4}{5}\right)(1-F) - (1-2\bar{q})(1-F)^3 + \left(\left(\frac{1}{5}\right) - \bar{p}\bar{q}\right)(1-F)^6\right]\alpha^2$$

Table 3 brings out the point that there is not very much difference in the results in the two cases. The values of $\overline{\sigma_w^2}$ differ by less than 2 percent for all values of q if F is as small as .10 and for values of q in a diagonal across the table from about q = .50 for small F to q = .10 for very large F. Intra-strain variance is, however, some 10 percent greater for small q, and F in the neighborhood of .50, in the case of progressive fixation, than in that of a steady state and the reverse is true for large q, and F in the neighborhood of .70. The reason is that for a given variance of gene frequencies and hence F, the distribution is more compact (platykurtic) where deviations from $\bar{q}$ tend to be reduced in proportion to their magnitude by crossbreeding (steady state) than where unimpeded. The variance of the character is maximum in strains in which q = .707. In a total population with low $\bar{q}$ and intermediate F, the proportion of the strains with gene frequencies that yield a high variance may be expected to be greater in the less compact distribution. The opposite situation holds where $\bar{q}$ is high and F rather high.

TABLE 1

The variance (σ_T^2) of a total population characterized by inbreeding coefficient F in the case of a dominant-recessive pair of alleles with unit difference in grade.

	F										
q	0	.10	.20	.30	.40	.50	.60	.70	.80	.90	1.00
.05	.0025	.0072	.0119	.0165	.0210	.0256	.0300	.0345	.0398	.0432	.0475
.10	.0099	.0186	.0272	.0356	.0439	.0520	.0599	.0677	.0753	.0827	.0900
.20	.0384	.0529	.0668	.0803	.0932	.1056	.1175	.1289	.1398	.1501	.1600
.30	.0819	.0957	.1146	.1296	.1437	.1570	.1693	.1808	.1914	.2012	.2100
.40	.1344	.1501	.1647	.1782	.1905	.2016	.2116	.2204	.2281	.2346	.2400
.50	.1875	.1994	.2100	.2194	.2275	.2344	.2400	.2444	.2475	.2494	.2500
.60	.2304	.2365	.2415	.2454	.2481	.2496	.2500	.2492	.2473	.2442	.2400
.70	.2499	.2499	.2490	.2472	.2445	.2410	.2365	.2312	.2250	.2180	.2100
.80	.2304	.2257	.2204	.2147	.2084	.2016	.1943	.1865	.1782	.1693	.1600
.90	.1539	.1482	.1424	.1364	.1303	.1240	.1176	.1109	.1041	.0971	.0900
.95	.0880	.0841	.0803	.0763	.0723	.0683	.0642	.0601	.0560	.0518	.0475
1.00	0	0	0	0	0	0	0	0	0	0	0

The decrease in total variance with increased F where the recessive allele is relatively abundant, referred to earlier, is shown in table 1. This holds for $q > (\sqrt{2(1-F)+F^2}-F)/2(1-F)$ and thus for $q > .707$ if F is close to 0, and for $q > .50$ if F is close to 1.

The increase in average intra-strain variance with increased F, where the recessive allele is relatively rare and F not too large (shown in table 2), depends largely on the increase in the mean with increase in F. There is no increase in mean in the absence of dominance, but a very pronounced one in the case of dominance and small $\bar{q}$. For very small $\bar{q}$, the intra-strain variance approaches $\bar{q}F(1-F)(1+4F)/(1+F)(1+2F)$ in the case of steady state,

TABLE 2

The average variance ($\overline{\sigma_w^2}$) within partially isolated subdivisions of a population in which a steady state (constant F) has been reached between the tendency toward fixation due to inbreeding and the opposed effect of occasional crossbreeding, for the same character as in table 1. The variance of the means of the subdivisions is the difference between corresponding entries in table 1 and 2.

	F (steady state)										
q	0	.10	.20	.30	.40	.50	.60	.70	.80	.90	1.00
.05	.0025	.0068	.0100	.0120	.0128	.0126	.0114	.0095	.0069	.0037	0
.10	.0099	.0174	.0226	.0254	.0262	.0252	.0225	.0185	.0133	.0071	0
.20	.0384	.0483	.0538	.0554	.0539	.0496	.0430	.0345	.0244	.0128	0
.30	.0819	.0885	.0898	.0869	.0806	.0717	.0605	.0475	.0329	.0170	0
.40	.1344	.1325	.1260	.1162	.1039	.0896	.0738	.0567	.0386	.0197	0
.50	.1875	.1734	.1571	.1395	.1208	.1016	.0818	.0617	.0413	.0208	0
.60	.2304	.2032	.1772	.1524	.1285	.1056	.0834	.0618	.0408	.0202	0
.70	.2499	.2122	.1793	.1502	.1238	.0997	.0773	.0563	.0366	.0179	0
.80	.2304	.1896	.1562	.1279	.1033	.0816	.0622	.0447	.0286	.0138	0
.90	.1539	.1234	.0994	.0797	.0632	.0492	.0369	.0261	.0165	.0079	0
.95	.0880	.0697	.0556	.0442	.0348	.0268	.0200	.0141	.0088	.0042	0
1.00	0	0	0	0	0	0	0	0	0	0	0

TABLE 3

The ratio of the average variance within completely isolated strains with unimpeded progress toward complete fixation, measured by successive values of F (ROBERTSON'S case) to the average variance within strains under conditions which yield the same values of F in steady states (of table 2).

	F									
$\bar{q}$	0	.10	.20	.30	.40	.50	.60	.70	.80	.90
.05	1.000	1.015	1.042	1.069	1.087	1.095	1.092	1.076	1.049	1.010
.10	1.000	1.012	1.034	1.056	1.072	1.079	1.076	1.063	1.039	1.004
.20	1.000	1.007	1.022	1.036	1.046	1.050	1.048	1.038	1.020	.994
.30	1.000	1.004	1.012	1.019	1.024	1.025	1.022	1.014	1.002	.984
.40	1.000	1.002	1.004	1.005	1.005	1.003	.999	.993	.984	.973
.50	1.000	1.000	.997	.993	.988	.983	.977	.972	.967	.963
.60	1.000	.998	.991	.983	.973	.965	.957	.953	.951	.954
.70	1.000	.996	.986	.973	.960	.948	.939	.935	.936	.944
.80	1.000	.994	.981	.964	.948	.933	.922	.918	.921	.935
.90	1.000	.993	.977	.957	.936	.919	.906	.902	.907	.926
.95	1.000	.992	.975	.953	.931	.912	.899	.894	.900	.921

with maximum at .46 which agrees with ROBERTSON's result for progressive inbreeding, although his formula $q[(4/5)(1-F)-(1-F)^3+(1/5)(1-F)^6]$ is quite different in appearance. The largest value of q at which there is an increase in $\overline{\sigma_w^2}$ with increase in F is .41 $(=\sqrt{1/6})$ in both cases, since in both, $\overline{\sigma_w^2}$ approaches $\bar{q}^2(1-\bar{q}^2)+F\overline{pq}(1-6\bar{q}^2)$ for very small F.

PROGRESSIVE INBREEDING: LIMITING CASE

While the form of the distribution of gene frequencies continually changes in the case considered by ROBERTSON, it approaches an almost rectangular distribution, $\phi(q) = 1$ between the limits 0 and 1, as F increases. The proportion of the strains that are heterallelic continually decreases at the rate $1/(2N)$ per generation as new strains drift into fixation at $q = 0$ or $q = 1$ (WRIGHT 1931). Let x and y be the proportional frequencies at $q = 0$ and $q = 1$, respectively, at a given time, leaving $1-x-y$ as the proportion still unfixed. The values of x and y must always be such that the mean gene frequency $\bar{q}$ is constant and the second moment about zero is related to F by the formula, $\mu_2' = q^2 + \overline{pq}F$

$$\phi(q) = \begin{cases} x & \text{for} \quad q = 0 \\ 1-x-y & \text{for} \quad 0 < q < 1 \\ y & \text{for} \quad q = 1 \end{cases} \tag{32}$$

The first and second moments about zero are as follows:

$$\mu_1' = y + (1-x-y)\int_0^1 q\,dq = y + (1-x-y)/2 = \bar{q} \tag{33}$$

$$\mu_2' = y + (1-x-y)\int_0^1 q^2\,dq = y + (1-x-y)/3 = \bar{q}^2 + \bar{p}\bar{q}F \tag{34}$$

From these

$$(35)\qquad \begin{cases} x = \bar{p} - 3\bar{p}\bar{q}(1-F) \\ 1 - x - y = 6\bar{p}\bar{q}(1-F) \\ y = \bar{q} - 3\bar{p}\bar{q}(1-F) \end{cases}$$

(36) Thus $\mu_4' = y + (1-x-y)\int_0^1 q^4 dq = y + (1-x-y)/5$

$$= \bar{q} - (9/5)\bar{p}\bar{q}(1-F)$$

$$(37)\qquad \overline{\sigma_w^2} = \mu_2' - \mu_4' = (4/5)\bar{p}\bar{q}(1-F)$$

This is the first term of the general expression arrived at by ROBERTSON by his wholly different mode of attack, and is the limiting value as F increases. It is 4 percent smaller than the corresponding limiting value, $\overline{\sigma_w^2} = (5/6)\overline{pq}(1-F)$ which the formula for the steady state takes when F is very close to 1.

OTHER COMPLICATIONS

The effects of incomplete dominance and overdominance in a system of partially isolated strains in a steady state can be analyzed similarly by use of the first four moments of $\phi(q)$. The formulae are in general more cumbersome than with complete dominance.

Recurrent mutation can be introduced into the concept of F without difficulty since its effect on gene frequency is like that of immigration. Letting u and v be the rates of mutation from and to the gene in question $\Delta q = v(1-q) - uq - m(q-\bar{q})$. It is merely necessary to let $a = 4N(m+u+v)$ to arrive at the same formulae as before in terms of $\bar{q}$ and F.

The introduction of selection into the concept is more difficult since selection tends to produce changes in gene frequency that are quadratic even with no dominance, and cubic with dominance in any degree, without considering the complications from the fact that selection operates on the genotype as a whole rather than on the separate loci. The formulae for $\phi(q)$ became unintegrable except by empirical means even in the simplest cases. If the state of balance is such that the standard deviation of $\phi(q)$ is small, an approximation can be obtained by using the best linear expression for Δq in the neighborhood of $\bar{q}$.

SUMMARY

The variance of a character, dependent on a completely recessive gene, in a population with partially isolated strains in which the tendency toward fixation due to inbreeding is balanced at a certain level of inbreeding by occasional cross breeding, is analyzed into the variance of strain means and the variance within strains. The formulae are in terms of gene frequency, inbreeding coefficient and gene effect.

The results are compared with those obtained by ROBERTSON in the case of subdivision into completely isolated strains that are tending toward fixation.

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APPENDIX

The conditions for a steady state with respect to the distribution of gene frequencies can be represented by a series of equations representing the persistence unchanged of each moment, after the occurrence of a systematic change and a random change. All moments obviously exist because of the finite range, 0 to 1. If all remain unchanged, the frequency curve does not change. The equation representing persistence of the n'th moment is as follows. The frequencies of q and δq are represented by $f(q)$ and $f(\delta q)$ respectively.

$$(38) \qquad \sum_{q=0}^{1} \sum_{\delta q=-q}^{1-q} [(q-\bar{q}) + (\Delta q + \delta q)]^n f(\delta q) f(q) = \sum_{q=0}^{1} (q-\bar{q})^n f(q)$$

Expanding the left hand member in powers of $(q-\bar{q})$ and $(\Delta q + \delta q)$, we note that the first term cancels the right hand member. Moreover, the following must hold for the *random* deviations: $\Sigma \delta q f(\delta q) = 0$, $\Sigma (\delta q)^2 f(\delta q) = \sigma_{\delta q}^2$, $\Sigma\Sigma [\delta q (q-\bar{q}) f(\delta q) f(q)] = 0$, $\Sigma\Sigma [\delta q \Delta q f(\delta q) f(q)] = 0$.

Unless $\sigma_{\delta q}^2$ is of the order of Δq or greater, the latter dominates so much that the distribution is practically restricted to the equilibrium value of q. The case of interest is that in which terms in $(\Delta q)^2$, $(\delta q)^4$, $(\Delta q)(\delta q)^2$ and higher powers may be treated as negligible. With this assumption the equations reduce to the following.

$$(39) \qquad \sum_{q=0}^{1} (q-\bar{q})^{n-1} \Delta q f(q) + \frac{n-1}{2} \sum_{q=0}^{1} [(q-\bar{q})^{n-2} \sigma_{\delta q}^2 f(q)] = 0$$

It is convenient at this point to substitute integration for summation, and $\phi(q)dq$ for $f(q)$, and represent $\Delta q\, \phi(q)dq$ by $d\chi(q)$

$$(40) \qquad \int_0^1 (q-\bar{q})^{n-1} d\chi(q) + \frac{n-1}{2} \int_0^1 (q-\bar{q})^{n-2} \sigma_{\delta q}^2 \phi(q) dq = 0$$

Integrating the first term by parts

$$(41)\qquad \left[\chi(q)(q-\bar{q})^{n-1}\right]_0^1 - (n-1)\int_0^1 \chi(q)(q-\bar{q})^{n-2}dq + \frac{n-1}{2}\int_0^1 (q-\bar{q})^{n-2}\sigma_{\delta_q}{}^2\phi(q)dq = 0$$

$$(42)\qquad \text{If } n=1,\ [\chi(q)]_0^1 = 0,\ \chi(1)=\chi(0)$$

$$(43)\qquad [\chi(q)(q-\bar{q})^{n-1}]_0^1 = \chi(1)[(1-\bar{q})^{n-1} - (-\bar{q})^{n-1}] = (n-1)\chi(1)\int_0^1 (q-\bar{q})^{n-2}dq$$

Equation (41) becomes

$$(44)\qquad \int_0^1 (q-\bar{q})^{n-2}[\chi(1)-\chi(q)+(1/2)\sigma_{\delta_q}{}^2\phi(q)]dq = 0$$

Thus *all* moments are the same before and after the occurrence of systematic and random processes if the following holds.

$$(45)\qquad \chi(q)-\chi(1) = (1/2)\sigma_{\delta_q}{}^2\phi(q)$$

$$(46)\qquad \frac{d}{dq}\log[\chi(q)-\chi(1)] = \left[\frac{d}{dq}\chi(q)\right]\Big/[\chi(q)-\chi(1)] = \frac{\Delta q\phi(q)}{(1/2)\sigma_{\delta_q}{}^2\phi(q)} = \frac{2\Delta q}{\sigma_{\delta_q}{}^2}$$

$$(47)\qquad \log[\chi(q)-\chi(1)] = \log(C/2) + 2\int(\Delta q/\sigma_{\delta_q}{}^2)dq$$

$$(48)\qquad [\chi(q)-\chi(1)] = (C/2)e^{2\int(\Delta q/\sigma_{\delta_q}{}^2)dq}$$

Equating the two expressions for $[\chi(q)-\chi(1)]$ of (45) and (48), we get the desired formula in which the constant C is such that $\int_0^1\phi(q)dq = 1$.

$$(49)\qquad \phi(q) = (C/\sigma_{\delta_q}{}^2)e^{2\int(\Delta q/\sigma_{\delta_q}{}^2)dq}$$

This is the general formula for the distribution of a gene frequency when a steady state has been reached.

PIGMENTATION IN A MOTTLED WHITE EYE DUE TO POSITION EFFECT IN DROSOPHILA MELANOGASTER

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THE phenomenon of phenotypic variegation in Drosophila, due to the translocation of a gene in a euchromatic region of the chromosomes to the neighborhood of a heterochromatic one, has been studied widely and interpreted in various ways (see BELGOVSKY 1946; EPHRUSSI and SUTTON 1946). After an analysis of one example of this variegation, the mottled white eye of the stock T(1;4)$w^{258\text{-}18}$ of *D. melanogaster*, DEMEREC and SLIZYNSKA (1937) suggested that proximity to heterochromatin may render the *w* locus unstable, so that it mutates from time to time to an allelic form, which may be reproduced and maintained for several cell generations, but which may in its turn mutate to another allele. The data indicate that, if this is so, the progression from one allele to another is not completely at random, but is restricted to a small number of the possible alleles of the *w* locus.

In T(1;4)$w^{258\text{-}18}$, the X chromosome has been broken just proximal to the *w* locus (following 3C4, salivary gland chromosome map) and the fourth chromosome in 101F. The distal end of the X, including the *w* locus, has been reattached to the stump of the fourth (chromocentral region) and the distal part of the fourth to the proximal part of the X.

According to DEMEREC and SLIZYNSKA, "on the eyes of homozygous 258-18 flies, cream, cherry and red colors . . . appear in definite patterns," and in their discussion the authors relate these colors to three alleles of the *w* locus, w^{cr} (cream), w^{ch} (cherry) and w^{+} (wild) respectively. In this paper, the cream color will be denoted as w^{cr} though no self-colored mutant at the *w* locus has been thus designated. It should not be confused with cream (*cr*), a series of modifiers of the eosin (w^{e}) allele of the *w* locus (BRIDGES and BREHME 1944).

If the mechanism of variegation is such as they postulate, one would expect, in a histological study of the eyes of these flies, to find three distinct types of ommatidia, whose pigmentation would correspond to that of the self-colored eyes of the mutants cream and cherry and of the wild type.

When I started to examine the eyes of T(1;4)$w^{258\text{-}18}$ flies histologically, I knew of only one published statement on the histology of white-mottled eyes. In one of his papers PANSHIN (1938) wrote to the following effect (translated from Russian): "The difference in pigmentation of facets depends on the fact that within one facet some cells have a normal quantity of pigment while the others have none." If this statement were found to hold true for the eyes of T(1;4)$w^{258\text{-}18}$ the hypothesis of DEMEREC and SLIZYNSKA would be

invalidated. It would be possible, moreover, on this basis to explain a considerable range of variation in pigmentation as seen from the surface of the eye; for one might expect the color to vary in intensity according to whether one or more layers of pigment cells were pigmented, and to appear opaque or transparent according to whether or not the upper layer of pigment cells was pigmented (MAINX 1938). In addition one might expect intermediate effects due to refraction, like that found by CASTEEL (1929) in a small area of an eye which, histologically, showed only normal pigmentation or complete lack of pigment.

PANSHIN, however, did not publish evidence for his statement, nor did he work with T(1;4)$w^{258\text{-}18}$, and it seemed of interest therefore to record in the present paper the results of a histological examination of the eyes of this particular stock. Subsequently, NOLTE (1950) has published some observations on the histology of the eye in white-mottled-4, referred to below.

In the course of work with T(1;4)$w^{258\text{-}18}$, an observation of a different sort was made. In the paper of DEMEREC and SLIZYNSKA it is affirmed that cherry or red spots on a cream-colored background are distributed at random over the eye. I found, on the contrary, that the major dark-pigmented areas are localized rather constantly in one part of the eye. This observation was confirmed in other stocks with mottled eyes. It has also been noticed by SCHULTZ (1941) who says: ". . . at low temperatures there is a small dark patch at the back of the eye . . . ." Some data relative to this phenomenon are presented here, in addition to the histological observations.

MATERIALS AND METHODS

All stocks of flies for this work were raised at about 18°C. A stock of T(1;4)$w^{258\text{-}18}$ was obtained from DR. DEMEREC at Cold Spring Harbor. The stock was carried by mating heterozygous females (T(1;4)$w^{258\text{-}18}/w$) with w males, but viable homozygous $w^{258\text{-}18}/w^{258\text{-}18}$ females and hemizygous $w^{258\text{-}18}$ males were obtained from this stock. The two latter types show patches of darker pigmentation on a cream-colored background, and also mottling for roughest (*rst*).

Eyes of T(1;4)$w^{258\text{-}18}/w$ females and T(1;4)$w^{258\text{-}18}$ males (which occur with a low frequency in the stock) were prepared in one of the following ways: (1) Whole flies or half heads fixed in absolute alcohol, passed through toluene series, imbedded in paraffin, serially sectioned, mounted on slides, cleared with xylol and mounted unstained in clarite or balsam. (2) Whole flies fixed by the freezing-drying method (GERSH 1948) infiltrated with paraffin and treated subsequently as in (1) with this difference—the sections were mounted on the slide by the dry method.

Eyes of males and females of six other stocks, namely Ore-R (Oregon-R, wild type), *bw* (brown), *v* (vermilion), w^{bf} (white-buff), w^{ch} (white-cherry) and w^{e} (white-eosin), were prepared in the same manner. In addition, some sections of Ore-R and *w* (white) eyes were examined after staining with Delafield's haematoxylin and eosin. All flies were aged at least two days after

emergence. Sections were made at 6 μ or 10 μ, and a few of the Ore-R eyes at 2 μ.

The study of the localization of the pigment in T(1;4)$w^{258\text{-}18}$ was supplemented by an examination of two other stocks—Dp(1;3)$N^{264\text{-}58}$ and mottled-28. The former is a stock homozygous for *w* which has in addition a small section of the X chromosome including the *w* locus inserted in the chromocentral region of the third chromosome. Flies of this stock have mottled eyes owing to the proximity of the *w* locus to heterochromatin, as in T(1;4)$w^{258\text{-}18}$. Mottled-28 (*mot-28*) is a recessive mutation at a locus near the centromere in the left arm of the third chromosome, with no detectable chromosomal aberration. The eyes are mottled with dark brown pigment and the stock is peculiar in that this pigment is formed even when flies are homozygous for white (*w/w; mot-28/mot-28*). It was in flies of this genotype that the eyes were studied. For further details of these stocks, see BRIDGES and BREHME (1944).

RESULTS AND DISCUSSION

The pigment cells in sectioned material

1. *General.* The histology of the adult wild type eye has been described by previous workers (*e.g.*, JOHANNSEN 1924; HERTWECK 1931). Figure 1 shows the structure of the individual ommatidium. In this diagram (A) are shown three layers of pigment cells: a distal layer of *primary pigment cells* (two in each ommatidium), a middle layer of *secondary pigment cells* (nine surrounding each ommatidium) and a proximal *post-retinal pigment cell* (probably one underlying each ommatidium).

The number of secondaries sheathing each ommatidium has been variously cited as from six to twelve. I agree with NOLTE (1950) that there are generally nine of these cells, although there often seem to be eight or ten owing to confusion with the cells of contiguous ommatidia.

There has been some doubt also as to whether the post-retinal pigment, lying below the basement membrane, is contained in a separate series of cells, or in processes extending through this membrane from the pigment cells above it (for discussion see NOLTE 1950). I am again in agreement with NOLTE that there is a layer of post-retinal pigment cells; in sections stained with haematoxylin and eosin, one can see their large nuclei with heavily staining nucleoli, and these nuclei correspond roughly with the overlying ommatidia (figures 13, 14). These cells contain granules similar to those in the primary and secondary pigment cells.

JOHANNSEN (1924) states that there is a fourth layer of *basal pigment cells,* and this was reaffirmed by PILKINGTON (1941) and NOLTE (1950), but HERTWECK (1931) was unable to convince himself that there were any such cells as distinct from the rudimentary eighth retinula cell which occupies a basal position in the ommatidium above the basement membrane. I have not found any nuclei between those of the eighth retinula cells and those of the post-retinal pigment cells.

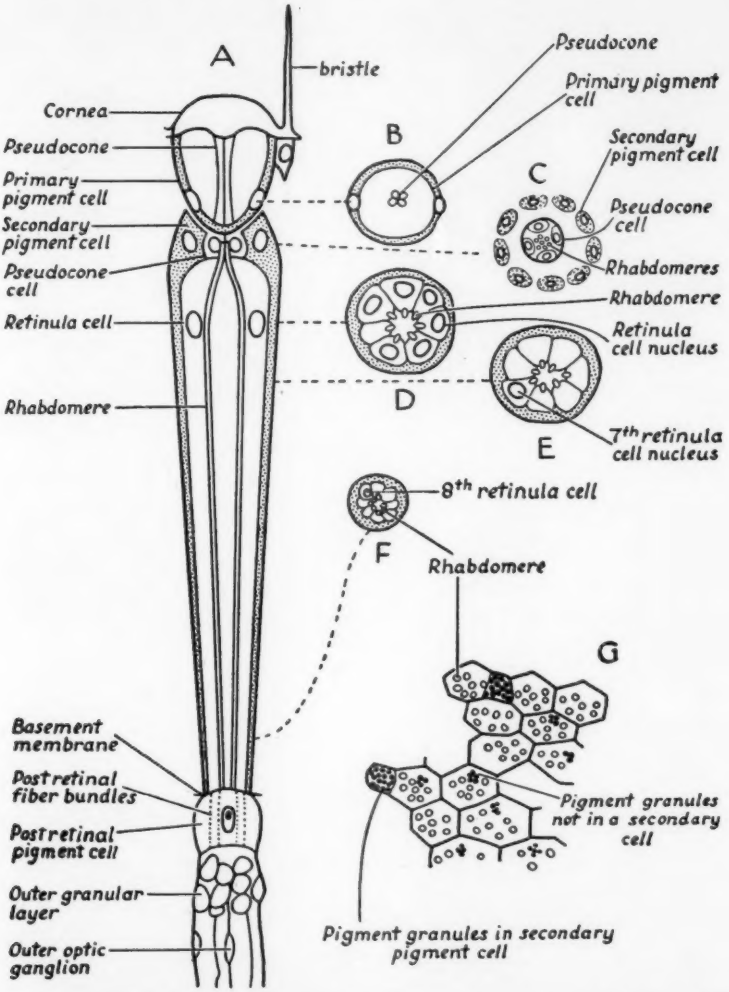
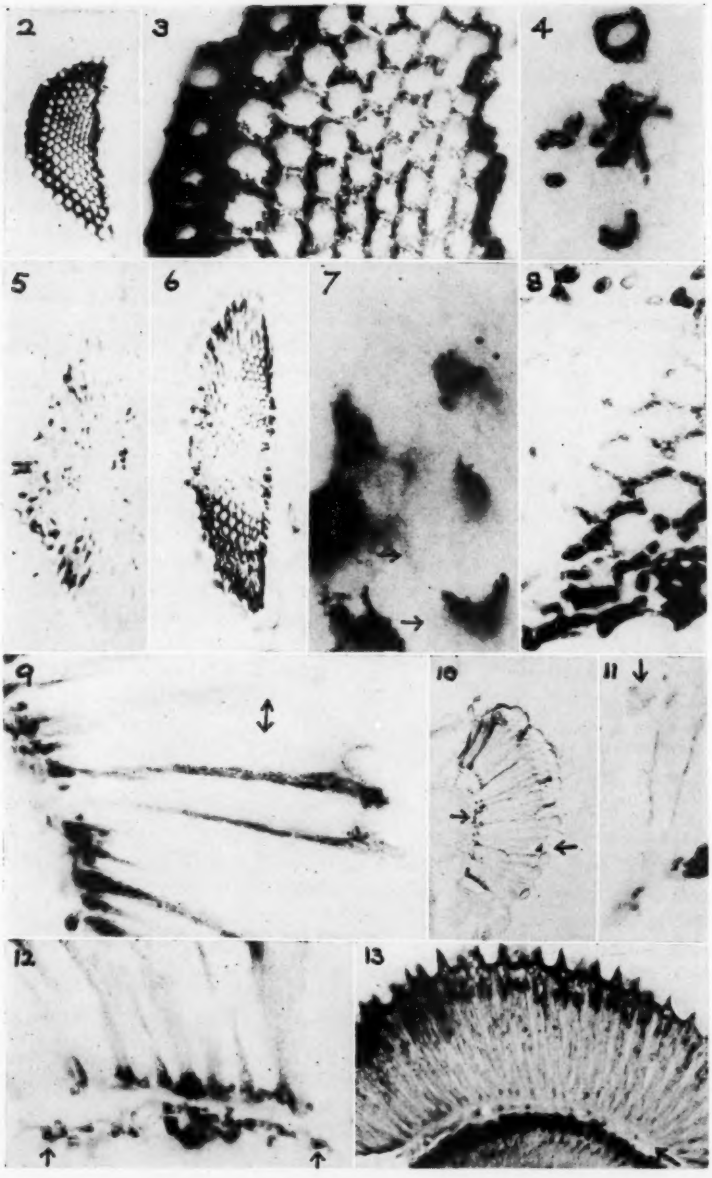


FIGURE 1.—A–F, diagrams of a single ommatidium to show location of pigment cells, for orientation purposes. A, longitudinal section; B–F, transverse sections at different levels. G, drawing of part of the eye in figure 5 to show small groups of pigment granules oriented with respect to the rhabdomeres. (A–F after HERTWECK 1931 with modifications; G, camera lucida, *ca.* × 680, drawn by MRS. E. B. PATTERSON.)



FIGURES 2–13.—Microphotographs of unstained sections through eyes of wild type Oregon-R (2, 3) and mottled white T(1;4)w^{ese-18} (4–12), and a stained section of a white eye (13).

There are two distinct pigments, the red and the brown (EPHRUSSI and HEROLD 1944). Both are present in both primary and secondary pigment cells, but MAINX (1938) found that the red predominates in the primaries and the brown in the secondaries. The pigments are confined to granules, and (again according to MAINX) the individual granules containing mainly the red pigment (as in *st* flies) appear in sections as "yellow," "ochre yellow," or "salmon red," while those containing mainly the brown pigment appear (as in eyes of *bw* flies) as "lilac red" (OSTWALD's Normenatlas, 1923). I consider those terms adequately descriptive of the colors of individual granules in the eyes of vermilion and brown flies respectively, whether fixed in alcohol or by freezing and drying. It is therefore necessary to bear in mind that in a histological description the colors of individual granules are not the same as those seen in a gross view of the surface of the eye; the reddish granules give a gross effect of brown, while yellowish granules give a gross effect of red.

In the wild-type eye the granules have been described as "yellow" or "golden-brown," and "wine-red," the former being concentrated in the distal region, especially in the primaries.

2. *Pigment cells in* $T(1;4)w^{258\text{-}18}$. In the eyes of flies of the $T(1;4)w^{258\text{-}18}$ stock, two types of primary pigment cells were observed. One type was pigmented with golden brown granules similar to those of the wild type eye but often more sharply demarked from, and contrasting more with, the underlying wine-red pigment of the secondaries. The other type was not visibly pigmented. A mosaic of the two types of cell is shown in figure 4, where the two primaries of one ommatidium (in transverse section) form an entire circle of

FIGURE 2.—Wild type, transverse section through ommatidia at level of secondaries, showing regular pattern formed by pigment cells. *ca.* × 140.

FIGURE 3.—Same as figure 2, × 680.

FIGURE 4.—Mottled white, transverse section through ommatidia at level of primaries; above, complete circle formed by two pigmented primaries; below, half-circle formed by one pigmented primary, the other being unpigmented. × 680.

FIGURES 5, 6.—Mottled white, transverse section through ommatidia of two different flies at level of secondaries. In these two eyes different proportions of pigment cells are fully pigmented, the remainder being either unpigmented or having pigment granules of an intermediate color. The two latter types are indistinguishable here. Compare with figure 2. *ca.* × 140.

FIGURE 7.—Same as figure 5, × 1300; arrows indicate position of cells with intermediate pigmentation.

FIGURE 8.—Same as figure 6, × 680. Compare with figure 3.

FIGURE 9.—Longitudinal section through ommatidia from same eye as figure 5; arrows indicate position of secondaries with intermediate pigmentation. × 680.

FIGURE 10.—Mottled white, longitudinal section through ommatidia; upper arrow shows pigmented post-retinal area underlying unpigmented secondaries; lower arrow, pigmented primary overlying unpigmented secondaries, and immediately below this, a pigmented secondary with corresponding primaries unpigmented. *ca.* × 140.

FIGURE 11.—Mottled white, longitudinal section through ommatidia, arrow indicates pigmented primary with corresponding secondaries unpigmented. Adjacent secondary pigmented. × 680.

FIGURE 12.—Mottled white, longitudinal section through bases of ommatidia; arrows show pigmented areas below basement membrane underlying unpigmented secondaries. × 680.

FIGURE 13.—White, longitudinal section through ommatidia stained with haematoxylin and eosin; arrow indicates post-retinal layer of pigment cells. × 415.

pigment, while in another ommatidium the pigment in a single crescent-shaped primary is contrasted with the lack of pigment in the cell which completes the circle.

The secondary pigment cells are of three types: (1) fully pigmented and comparable to those of the wild type fly; (2) with no visible pigment; and (3) very slightly pigmented, densely packed with granules of a pale yellowish pink color. The third type was identified in eyes fixed by freezing and drying, but not after alcohol fixation. As in the case of the primaries, the individual ommatidium may be a mosaic, with secondaries of two or even three different kinds. The cells with intermediate pigmentation are not confined to the immediate neighborhood of those with wild type pigmentation, but are found, mingled with colorless cells, at a distance of several ommatidia away from any wild-type secondary.

Figures 5 to 9 show various aspects of the pigmentation of the secondaries. Figures 5 and 6 are of contrasted eyes with, respectively, very few and rather many darkly-pigmented cells. Figures 6 and 8 should be compared with figures 2 and 3, which are photographs of a wild type eye at the same magnifications. The intermediate type of secondary does not show well in photographs, but the location of such cells is indicated in figure 7, and in the longitudinal section of the ommatidia in figure 9.

In the unpigmented cells no granules were visible, but a slide stained in iron haematoxylin showed that granules are present in all pigment cells. There is, however, variation in the stainability of the granules which corresponds roughly with the initial degree of pigmentation. This was determined by making camera lucida drawings to show the pigmented areas before the slide was stained with haematoxylin. After staining it was found that cells in the most strongly pigmented region (dorsal part of the eye) contained very dark brown to black granules. In isolated small patches of fully pigmented cells in the more ventral region the granules varied from pale brown to black, while in all other pigment cells they were very pale. There was no noticeable difference in granule size or density in different cells.

In some eyes there were regions where pigment was present below the basement membrane (see figure 1 A) although there was no pigment surrounding the ommatidia immediately above this membrane (figures 10 and 12). A study of sections stained with haematoxylin and eosin confirmed NOLTE's (1950) view that this pigment is contained in separate post-retinal cells (figures 13, 14). The pigment in these cells is usually very dense, like that of wild type, but there are a few intermediate and colorless cells like those in the secondaries.

Transverse sections through the proximal parts of one eye showed a small patch of pigment in many ommatidia which were otherwise unpigmented. This patch was always located in the same position within the ommatidium, close to the rhabdomeres and between corresponding rhabdomeres (which form a pattern repeated from one ommatidium to the next); and it was much smaller in cross section than a secondary pigment cell at the same level (figure 1 G). It seems possible that this pigment is contained in the rudimentary eighth retinula cell (figure 1 F), which was first described by DIETRICH (1909).

In sections of many of the eyes examined, the pigment cells of ommatidia in the dorsal region were all strongly pigmented. Ventrally, isolated ommatidia or groups of ommatidia contained some fully pigmented cells (figure 10). In this region it is possible to find ommatidia in which the primaries are pigmented and the secondaries are not (figures 10, 11) and *vice versa* (figure 10). Pigmentation of primaries and secondaries thus seems to be independent (in contrast, apparently, to white-mottled-4, where NOLTE states that unpigmented sectors extend from the post-retinals to the primaries), but in areas where the secondaries are uniformly pigmented throughout many ommatidia, the primaries tend likewise to be uniformly pigmented. This will be referred to again in the following section.

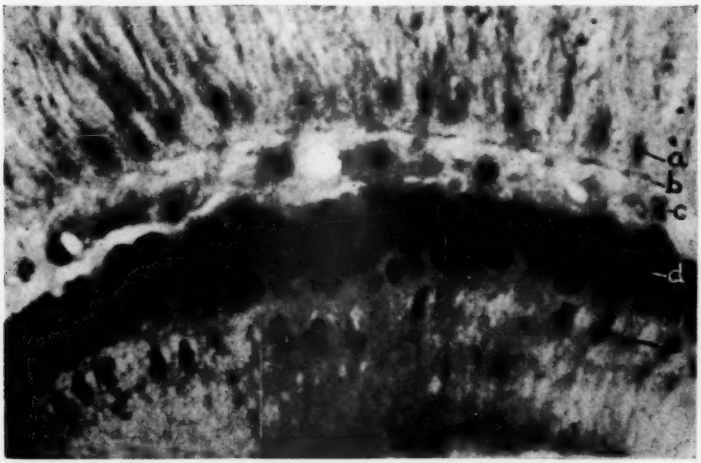


FIGURE 14.—Longitudinal section through bases of ommatidia of a white eye stained with haematoxylin and eosin (same section as figure 13). a, layer of eighth retinula nuclei; b, basement membrane; c, fenestrated zone, with tracheae (lacunae) and nuclei of post-retinal pigment cells; d, layer of monopolar neurons; e, nuclei of external optic glomerulus. × 1700.

To sum up, pigmentation can vary from cell to cell, and is not necessarily uniform within a whole ommatidium; but it is not, for the secondary and post-retinal pigment cells at least, a simple all-or-none effect, for a third, intermediate type occurs. This again contrasts with NOLTE's finding of an all-or-none effect in white-mottled-4.

3. *Pigment cells in some intermediate alleles of the w locus.* White-buff (w^{bf}). This mutant was chosen because it is one of the palest in the series of white alleles and may be considered as comparable in that respect with the cream background of the white-mottled eyes described above. In gross view the eyes of males are slightly lighter than those of females; in sections they are indistinguishable. The basal pigment cells are packed with granules, but they are colorless or almost so. Numerous colorless granules are also present in the

secondaries, but at the apex of these cells a few bright yellow granules are clearly distinguishable. There are also a few such granules in the primaries.

White-cherry (w^{ch}). This mutant was studied by way of comparison with the intermediate (cherry) pigmentation of the white-mottled eye in T(1;4)$w^{258\text{-}18}$. In males there is a very dense concentration of orange-pink granules in the post-retinals and at the bases of the secondaries. The granules are less numerous in the distal parts of the secondaries and in the primaries, where they are mostly golden-brown. In females (whose eyes appear darker than those of males in gross view) the granules of the post-retinals are pinker and less orange than those in the males. In other respects males and females are similar. All granules in cherry eyes are more intensely pigmented than the "intermediate" of the white-mottled eye.

White-eosin (w^e). In males there are many pale pink granules in the post-retinals. They are more intensely pigmented than the "intermediates" of the white-mottled eye. The secondaries contain a very sparse sprinkling of browner granules, and the primaries are thinly pigmented in a similar manner. In females (whose eyes are darker in gross view) the only difference is that the pigment granules in the post-retinals are an intense orange-red.

White (w). It may be mentioned here that in sections of w eyes stained with haematoxylin and eosin, the pigment cells are seen to be as densely packed with granules as in wild type eyes similarly treated.

4. *Discussion.* A comparison of the intermediate secondary pigment cells found in T(1;4)$w^{258\text{-}18}$ with the secondaries of the intermediate mutants at the w locus described above reveals two marked differences. First, the individual granules in the intermediate cells of the white-mottled eye are much paler than those in any of the mutants studied (except for the post-retinal granules in white-buff). Second, the number of pigmented granules in the secondaries of the white-mottled eye does not appear to be reduced, while in the mutants examined there is an obvious reduction in the number of pigmented granules in the secondaries. Thus, the pigmentation of the cherry eye and the similar coloring of the cherry patches in the white-mottled eye are apparently produced in different ways: the former, largely by reduction in the number of pigmented granules as compared with wild type; and the latter, by reduction in the amount of pigment per granule as compared with wild type.

While the histological picture in T(1;4)$w^{258\text{-}18}$ seems at first sight to be consonant with the hypothesis of mutability as a result of change in position of the w locus, the comparison with mutant alleles at the w locus gives the impression that the changes that occur within the white-mottled eye are of a different kind from the mutational change from w^+ to a lower allele, or *vice versa.*

5. *Advantages of fixation by freezing and drying.* Some of the results obtained in the course of this work show clearly the superiority of the freezing-drying method for this type of study.

For instance, it has been noted that the intermediate cells in the white-

mottled eye were not detectable after fixation with absolute alcohol, though they were after freezing and drying. This is undoubtedly because the small amount of pigment in the granules of these cells is dissolved out during even a short period in alcohol. It is conceivable that intermediate cells could also be demonstrated in white-mottled-4 with the freezing-drying technique (see NOLTE 1950).

Again, MAINX (1938) did not detect any colored granules at the distal ends of the ommatidia in white-buff, but these are very clear in the frozen-dried white-buff eyes. This discrepancy may be due in part to a genetic reduction in the amount of pigment in the w^{bf}; *bw* as well as in the w^{bf}; *st* eyes which MAINX examined. The distal pigment is much more striking than the proximal, though, and it seems unlikely that it would be reduced to a greater extent, except by solution during alcohol fixation (MAINX avoided the use of water in mounting sections). NOLTE's (1950) observations on pigmentation of the wild-type eye indicate that pigment is more readily lost by solution from the distal parts of the eye than from the proximal.

Finally, although some shrinkage and consequent distortion occurs during freezing and drying, it is far less than during alcohol fixation. The superiority of freezing-drying in this respect can be seen by comparing figure 14 of this paper with NOLTE's (1950) figure 4, from material fixed in modified Carnoy. In the latter, distortion is particularly noticeable in the fenestrated zone immediately below the basement membrane and in the region below the nuclei of the external optic glomerulus. The basement membrane stains with haematoxylin (see figure 14 in which it shows some slight discontinuities), and I believe that shrinkage in NOLTE's material has disrupted the membrane into discrete parts which can be mistaken for nuclei. The " basal nuclei " seen in cross or oblique sections (NOLTE's figure 3) cannot, of course, be accounted for in this way, but may conceivably be the bases of the rhabdomeres, which also stain strongly with haematoxylin.

The gross pigmentation pattern of the eye

In order to study the localization of pigment, the surface of the eye was arbitrarily divided into six regions, as shown in figure 15 A. In the flies examined, both eyes were scored for presence or absence of a major dark-pigmented area (one-quarter of a region or more) in each of these regions. The results, expressed as percentage of eyes with dark pigmentation in a given region, are shown in figure 15 B–E.

At 18°C, the T(1;4)$w^{258\text{-}18}$ males show a strong localization of pigment in middle-caudal (4) and upper caudal (2) regions. Sixteen out of seventy flies had dark pigment restricted to region 4 in both eyes; thirty others had one eye in which the pigment was so restricted. Eight exceptional eyes lacked pigment in region 4, but it was then present in region 2 or (in one eye) 6.

In heterozygous females, T(1;4)$w^{258\text{-}18}$/*w*, the pigment is more generally distributed, chiefly in the caudal regions 2, 4 and 6. Only five flies out of sixty

had the pigment restricted to region 4 in both eyes, seventeen in one eye. Six eyes lacked pigment in region 4, though it was present in region 2 or in 2 and 6.

In the Dp(1;3)$N^{264\text{-}58}$ stock, homozygous for white, the darkly-pigmented area is usually larger. Males and females, both of which may be either homozygous or heterozygous for the w^+ duplication, appear to have similar eyes, but only females were scored. Again region 4 is most frequently pigmented. Only one fly out of twenty-five had this area lacking in pigment in both eyes, and five in one eye. Here again, 2 or 6 was pigmented if 4 was not, except in two eyes where no large dark spot occurred.

Mottled-28, a stock in which the mottling is apparently of an entirely different type, showed a dorsal shift in the location of the pigment, which was chiefly in region 2, but also frequently in region 1 or 4. One fly out of ten

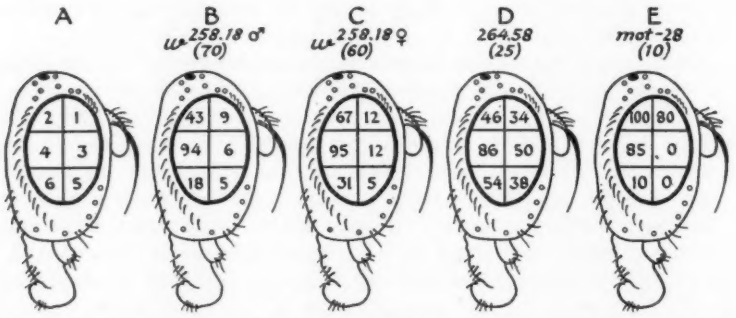


FIGURE 15.—Diagram of right eye of Drosophila, showing A, regions 1–6 used in mapping distribution of pigment; B–E, distribution of pigment in eyes of four different groups of flies, as shown by percentage of eyes having major pigmented areas in each of the regions 1–6. Total number of eyes scored given in parenthesis under name of stock. Drawn by MRS. E. B. PATTERSON.

lacked pigment in region 2 in one of its eyes, which was pigmented only in region 4. Eyes of males and females are similar.

A cross was made between T(1;4)$w^{258\text{-}18}$ males and females from an inbred stock of brown scarlet (*bw; st*, 18 generations of brother-sister matings). F_1 females, heterozygous for the translocation and for *bw* and *st*, were backcrossed to *bw; st* males and the male progeny of this backcross was examined, and some individuals mated again with *bw; st* females to test their genotype with regard to *bw* and *st*. In this male progeny, T(1;4)$w^{258\text{-}18}$ males of the classes $bw^+; st^+$, *bw/bw* and *st/st* all showed localization of pigment, while T(1;4)$w^{258\text{-}18}$; *bw/bw; st/st* males had uniform and almost colorless eyes. This shows that both the red and brown pigments are affected, and confirms the observation in sectioned material that where one type of pigment cell is mainly pigmented, so too is the other type. The same conclusion was reached by CHEN (1948): "Dans les deux cas ($w^{258\text{-}18}$; *bw* and $w^{258\text{-}18}$; *st*) le mode de panachure est le même que chez le mutant $w^{258\text{-}18}$."

Now, we can assume that mutation is the cause of differences in pigmentation in different regions of the mottled white eye, and explain the observed pattern in this way. In the upper caudal region, mutation from w^{cr} to w^{+} can occur at an earlier or later stage in the development of the eye, and in the former event the w^{+} allele remains stable throughout several cell generations giving rise to a large wild type spot (although in a related stock of $T(1;4)w^{258\text{-}18}$, apparently differing only in certain genetic modifiers, DEMEREC and SLIZYNSKA concluded that the w^{+} allele was unstable, mutating to w^{ch} and w^{cr}). In the rostral and ventral regions, on the other hand, mutations of the w^{cr} allele occur only at the later stages, giving rise to the small patches of ommatidia in which some or all of the pigment cells show more intense pigmentation. In other words, some property of the upper caudal region favors mutation of the w^{cr} allele especially at early stages in the development of the eye.

There are some objections to this explanation. It assumes that the size of darker spots on the cream background depends on cell lineage and on the stage of development at which a mutation occurs. CHEN (1948) has shown that the number of dark-pigmented facets can be modified by temperature changes during the pupal stage, and it is also known (DEMEREC 1950) that all, or nearly all of the ommatidial precursors are laid down by the last day of larval life. If at higher temperatures the size of the dark spots is increased (and the extent of the dorso-caudal pigmented area certainly is increased) the greater size of a spot cannot be correlated with a greater number of cell generations in which its ommatidia were derived from a single initial ommatidium or cell, because new ommatidia are not being formed during this pupal temperature sensitive period. The addition of new wild type or cherry facets under the influence of high temperature during the pupal stage must then be assumed to be due to independent mutations from w^{cr} within individual ommatidia during their development from the four-cell precursor stage to complete differentiation. Moreover, as the temperature effective period seems to extend to the last day of pupal life, when pigment formation has already started, " mutations " which alter pigmentation at this stage would presumably occur independently of cell division, which is unlikely to be taking place at this time.

According to CHEN, temperature changes are ineffective during the larval period, so that changes from w^{cr} toward wild-type apparently do not occur except during pupal, and possibly during embryonic, stages. There are some data (J. W. GOWEN, personal communication) indicating that there is a temperature sensitive period, for some white-mottled stocks at least, very early in development. Very early changes may, therefore, produce a pattern determined by cell lineage; but when the changes at the pupal stage are superimposed on the former, a final pattern of pigmentation which is not entirely dependent on cell lineage will be the result.

Another difficulty arises in connection with the direction of change. In accordance with DEMEREC and SLIZYNSKA, this discussion has assumed that in the light background stock of $T(1;4)w^{258\text{-}18}$ used in this work w^{cr} changes

to w^+, with or without w^{ch} as an intermediate stage. The work of DUBININ and SIDOROV (1935) and PANSHIN (1935) provides some evidence that in position effects associated with a break in heterochromatin the affected gene retains, in all essentials, the form it had before rearrangement; in other words, a wild type allele remains wild type and can, theoretically, be re-extracted in the normal form by crossing over into a normal chromosome. $T(1;4)w^{258\text{-}18}$ was obtained by irradiating a w^+ male. If it is assumed that the darker spots in the eye are due to changes from w^{cr} to w^{ch} or w^+, it seems necessary also to assume an initial change very early in ontogeny from the original w^+ allele to w^{cr}. It is hardly possible to suppose that this change occurred as a mutation at the time of irradiation, because DEMEREC and SLIZYNSKA report that light and dark background stocks were derived from the original one by selection, and in the dark background stock they postulate that the direction of change is from w^+ towards w^{cr}. In the light stock, then, the direction of mutation (if such it is) must be reversible, so we might expect, at least occasionally, to find one or more cream facets within a darker spot. The absence of such observations can only be explained by assuming that change from w^+ to w^{cr} is confined to an early embryonic stage, while changes from w^{cr} to w^+ can occur subsequently. In the dark stock, on the other hand, changes from w^+ to w^{cr} can presumably occur during pupal life, and changes from w^{cr} to w^+ not at all.

These arguments are not conclusive, but they indicate the difficulty of presenting a consistent hypothesis in terms of mutability, *sensu stricto,* as an explanation of the variegated phenotypes found in a large group of position effects.

As has been seen, the histological picture suggests that the type of pigmentation found in a white-mottled eye is different from that of the mutant alleles of the *w* locus.

The gross pattern of variegation in the eye may be determined by some developmental factor other than mutation. The preferential localization of pigment in regions 2 and 4 sems to be a general phenomenon which is manifested independently of the existence of a position effect; for it occurs also in the *mot-28* stock, in which there is no demonstrable position effect. It may also be relevant that in another position effect, associated with In(2LR)40d, the abnormality of a deeply pigmented crust forming on the surface of the eye occurs preferentially in the ventral part of the eye (HINTON 1949); while in several mutants that affect the structure of the eye, the ventral half is more severely affected than the dorsal (*e.g.,* in almondex, Deformed, Kidney, Lobe). It is not probable that somatic mutation is involved in all these cases, but a common factor might affect gene mutation in the position effects, and some other stage between gene and end-result in the mutant stocks; or a common effect on the *action* rather than the essential structure of the gene seems a possible explanation of the facts.

It is rather striking, in connection with the localization of pigment, to find the following passage in UMBACH'S (1934) description of the development

of pigment in the eye of the mealmoth, *Ephestia Kühniella* Z.: "Bei der Pigmentbildung in den Retinulazellen ist ebenso wie für die Formwandlungsprozesse der dorsocaudale Bezirk Differenzierungsmittelpunkt. Tägliche Beobachtung unter dem Binokular ziegt, wie in den ersten 10 Tagen der Puppenzeit ein 'Pigmentstrom' über die Augenanlage hinzieht. Die Pigmentierung schreitet ventralwärts schneller als in rostraler Richtung fort. . . . Am 10 Tage nach der Verpuppung erscheint die gasamte Fläche der Augenanlage durch Pigment verdunkelt." UMBACH adds that pigmentation of the ommatidia is independent of their stage of development, some becoming pigmented at an early and others at a later stage of differentiation, according to their position within the eye.

Is it merely a coincidence that in some Drosophila eyes the pigment is confined to approximately the same region where it first begins to develop in the eyes of Ephestia?

In Drosophila, those who have studied the development of the eye (*e.g.*, KRAFKA 1924; BODENSTEIN 1938; STEINBERG 1943) have observed no regional differences in the time of onset of ommatidial differentiation; nor is there evidence that pigmentation of the ommatidia is other than simultaneous. In fact, the retinula cells, which alone show the "Pigmentstrom" in Ephestia, remain unpigmented in Drosophila.

In order to determine more certainly whether pigmentation in Drosophila occurs simultaneously over the whole eye or not, flies were raised at a low temperature (about 15°C) to slow down development. In the conditions of the experiment, puparium formation started about fifteen days after the culture was started (with non-virgin parents), and adult flies emerged about thirteen days from puparium formation. Some time after the sixth day (not accurately timed) from puparium formation, the earliest stage of pigmentation that could be seen was in the form of a very pale yellow color distributed uniformly over the whole surface of the eye.

Thus there seems to be no obvious relationship between the localization of pigment in Drosophila and the site of its initial formation, and some other factor must be sought for.

It might be postulated that the localization of pigment is related to a gradient which is established within the eye before the beginning of pigment formation, and which is effective in the white-mottled eye (though not in the wild-type eye nor those of most other mutants) by virtue of a change in the sensitivity of the pigment cells to the factor which determines the gradient. Suppose, for instance, that a diffusible substance were at the highest concentration in the dorsocaudal center, diminishing to a lower concentration peripherally; and that pigment formation could proceed wherever the concentration reached a threshold inversely related to the sensitivity of the individual pigment cell. In the wild type eye it could be assumed that both concentration and sensitivity are high enough in all parts of the eye to ensure uniform pigmentation. If in the white-mottled eye the pigment cells tended to be less

sensitive, owing to modification of the w^+ locus, then we might expect pigment formation to be normal in the area of highest concentration and elsewhere to be limited to those cells whose sensitivity was normal or nearly so. This would result in the pattern of a dorsocaudal pigmented area with smaller peripheral spots. Sensitivity could conceivably vary discontinuously, depending on discontinuous states (temporary modifications, not strictly mutant states) of the w^+ locus, and thus account for the intermediate type of pigmentation. The effect of high temperature could presumably be either to increase the sensitivity of the pigment cells or to reduce the steepness of the gradient (for instance, by increasing the rate of diffusion). Where temperature changes are effective in very early stages of the life cycle, the former mechanism would operate, and during the pupal temperature sensitive period the latter might also come into play.

A diffusion gradient is not, of course, the only form which can be invoked to explain the pattern of pigmentation. Many other factors could play a similar role. One such factor which may influence pigmentation has been mentioned by BODENSTEIN (1943) who relates that in the development of wild type eye-discs transplanted (together with ring-glands) into adult hosts, " we observe most frequently that only certain eye regions are pigmented while others are still white," though he does not give further details as to which regions are preferentially pigmented. He suggests that this phenomenon is due to the position of the graft in the host, and more directly to local differences in oxygen supply. It is possible that such differences occur also in normal development of the eye, and are significant only for certain genotypes.

SUMMARY

The structure of the ommatidium in *D. melanogaster* is discussed.

In the mottled eye of $T(1;4)w^{258\text{-}18}$ the individual secondary pigment cells appear to be fully pigmented, unpigmented or intermediate. The post-retinal pigment cells are mostly fully pigmented, but may be unpigmented or intermediate. Only two types of primary pigment cells, fully pigmented and unpigmented, were distinguished.

The intermediate pigmentation in $T(1:4)w^{258\text{-}18}$ differs histologically from that of three intermediate alleles of the white locus. It is argued that the position effect is not due to mutation, in the strict sense, at a locus that has become unstable.

Further histological details of the pigmentation in $T(1;4)w^{258\text{-}18}$ are described.

The gross pattern of pigmentation, as seen at the surface of the eye, is not entirely random, pigment being most frequently concentrated in the dorsal caudal region of the eye. The size of this pigmented area does not seem to be a function of its cell lineage. It is suggested that the pattern may be due in part to a developmental factor influencing the manifestation of the position effect, rather than being fundamental to the mechanism of position effect.

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